Ť. - Ť.

Approved for Release: 2015/01/05 C05184036

SECRET

USE OF TOXINS AND OTHER LETHAL CHEMICALS IN SOUTHEAST ASIA AND AFGHANISTAN

16 February 1982

This document is UNCLASSIFIED after NFIB approval.

SECRET

Approved for Release: 2015/01/05 C05184036

UNCLASSIFIED

ĩ

3

CONTENTS

	Page
KEY JUDGMENTS	1
Laos Kampuchea The Soviet Role in Southeast Asia Afghanistan	1 1 1 1
DISCUSSION	3
Background. Methodology. Laos. Kampuchea. Afghanistan. What Chemical Agents Are Being Used? Soviet Chemical Warfare Activities.	3 4 5 8 10 12 14
CONCLUSIONS	17
ANNEXES	
 A. Tabulations of Reported Chemical Warfare Attacks B. A Lao Pilot's Story C. US Army Surgeon General's Investigative 	A-1 B-1
D. Analysis and Review of Tricothecene Toxins E. Medical Evidence	D-1 E-1

UNCLASSIFIED

_Approved for Release: 2015/01/05 C05184036____

UNCLASSIFIED

KEY JUDGMENTS

Laos

Lao and Vietnamese forces, assisted by Soviet logistics and supervision, have used lethal chemical agents against H'Mong resistance forces and villages, causing thousands of deaths since at least 1976. Trichothecene toxins have been positively identified as one of the classes of agents used, but medical symptoms indicate that irritants, incapacitants, and nerve agents also have been employed.

Kampuchea

Vietnamese forces have used lethal trichothecene toxins on Democratic Kampuchean troops and Khmer villages since at least 1978. Again, medical symptoms indicate that irritants, incapacitants, and nerve agents also have been used.

The Soviet Role in Southeast Asia

The one hypothesis that best fits all the evidence is that the trichothecene toxins were developed in the Soviet Union, provided to the Lao and Vietnamese either directly or through transfer of technical know-how, and weaponized with Soviet assistance in Laos, Vietnam, and Kampuchea. There is no intelligence at hand to support alternative explanations, such as completely independent manufacture and use by the Vietnamese. It is highly probable that the USSR also provided other chemical warfare agents. While the evidence on the Soviet role does not constitute proof in the scientific sense, the Intelligence Community finds the case to be thoroughly convincing.

Afghanistan

Soviet forces in Afghanistan have used lethal and casualtyproducing agents on Mujahedin resistance forces and Afghan villages since the December 1979 invasion. There is some

Note: This paper is Unclassified.

Ŧ

7

evidence that Afghan Government forces may have used chemical weapons provided by the USSR against the Mujahedin even before the invasion. No agents have been identified through sample analysis, but we conclude from analysis of all the evidence that attacks have been conducted with irritants, incapacitants, nerve agents, phosgene oxime, and perhaps trichothecene toxins, mustard, lewisite, and unidentified toxic smokes.

UNCLASSIFIED

DISCUSSION

Background

1. In September 1981 the US Government in a public declaration raised the probability that the trichothecene class of toxins*--poisonous chemical substances extracted from biological material (specific molds)--was the mysterious lethal agent that had been used for many years in Laos and Kampuchea. This significant statement was precipitated by the discovery of high levels of trichothecene toxins in a vegetation sample collected shortly after a March 1981 Vietnamese chemical attack in Kampuchea. That public declaration, however, rested on a much broader base of evidence than the analysis of that sample.

2. In April 1980 the Intelligence Community had already concluded that lethal agents had almost certainly been used against H'Mong tribespeople in Laos. There was less certainty about the use of lethal agents in Kampuchea, mainly because of suspicions about the propaganda campaign of Pol Pot's Democratic Kampuchean (DK) forces. The DK claims were subsequently shown to be valid. It was also concluded that chances were about even that lethal agents had been used in Afghanistan. There was little doubt by this time that riot control agents and some form of incapacitants had been used in all three countries. Since mid-1980, sufficient additional evidence has allowed a much firmer Intelligence Community judgment than that stated in the April estimate. There is now no doubt that deaths and casualties have resulted from chemical attacks in all three countries.

3. Analysis of additional samples from Laos and Kampuchea has revealed at least four trichothecenes, further supporting our conclusion that toxins were used. A review of all the reports indicates the use of many different chemical agents, means of delivery, and types of chemical attacks. In some cases, the symptoms are typical of those caused by trichothecenes, but in many cases the symptoms suggest other agents, which we have not been able to identify

3

^{*} Trichothecene toxins, like all other toxins, are chemical compounds derived from biological material. For purposes of this assessment, toxins are characterized as chemical warfare agents. Their manufacture, however, would most likely take place in biological warfare facilities, even if the toxins were synthetically produced.

through sample analysis. Significant differences as well as similarities have surfaced in the reports from the three countries. The evidence from each country, therefore, is described separately, with attention drawn to similarities where appropriate.

Methodology

4. The intelligence judgments of this study were arrived at through the following analytic process:

- -- Every relevant piece of information on reported chemical warfare incidents was reviewed, recorded, and tabulated (see annex A). Numbers of attacks and deaths were screened for potential duplication. An extensive data base on the Soviet chemical and biological warfare program was also searched.
- -- All the physical evidence available to the US Government--including environmental samples and background controls--was reviewed (see annex D).
- -- A scientific report on toxins was prepared, including the analysis leading to the conclusion that trichothecenes were probably among the agents used in Southeast Asia. The report also documents the extensive toxin research conducted in the USSR (see annex D).
- -- An analysis of the medical evidence was prepared, drawing on all available information from Southeast Asia and Afghanistan (see annex E). This incorporated the findings of the Department of Defense medical team (see annex C), which concluded that at least three types of agents were used in Laos.
- -- Extensive consultations were held with government and nongovernment scientists and medical authorities, many of whom were asked to review our evidence. Experts from other countries were also consulted.

5. After the data base was organized to permit comparative analysis, the study focused on three separate questions:

4

UNCLASSIFIED

- -- Have lethal and other casualty-producing agents been used in Southeast Asia and Afghanistan?
- -- What are these agents and how and by whom are they employed?
- -- Where do these agents originate and how do they find their way to the field?

6. Although the evidential base differs for each country, the analytic approach used was the same. The testimony of eyewitnesses--date, place, and type of attack--was matched against information from defectors, journalists, and international organizations and sensitive information that often pinpointed the time and place of chemical attacks. In addition, the intelligence files on military operations in the areas where chemical attacks had been reported were searched to establish whether air or artillery strikes took place or whether there was fighting in the areas where chemical agents were reportedly used. In all three countries, we identified a number of instances in which eyewitness accounts could be directly correlated with information from other sources.

There is no evidence of any systematic propaganda 7. campaign having been mounted by the H'Mong or the Afghan resistance forces to promote the allegation that chemical agents have been used on their people. Rather it was the US Government, other governments, and private individuals from many countries that publicized the use of chemical agents and that provided the evidence to international organizations. On the other hand, there were early indications that Pol Pot's Democratic Kampuchean resistance did engage in an organized propaganda campaign on chemical agent use. These indications made us very cautious about accepting DK allegations, which increased markedly after the chemical attacks in Laos were publicized. For Kampuchea, therefore, we were particularly insistent in our efforts to confirm allegations made with sources of information that in no way could be part of a propaganda or deception campaign.

Laos

8. Reports of chemical attacks in Laos date from the summer of 1975 to the present. These reports describe 261 separate attacks in which at least 6,504 deaths were cited as having resulted directly from exposure to chemical agents.

5

The actual number of deaths is almost certainly 9. much higher, since the figure above does not take account of deaths in attacks for which no specific casualty numbers The greatest concentration of reported use were reported. of chemical agents occurred in the area where the three provinces of Vientiane, Xiangkhoang, and Louangphrabang adjoin (see map, figure 1). This triborder region accounted for 77 percent of the reported attacks and 83 percent of the chemical-associated deaths. Most of the reported attacks took place in 1978 and 1979. In the past two years, the incidence of chemical attacks appears to have been lower, but reported death rates among unprotected and untreated victims higher--only seven chemical attacks were reported in the fall of 1981, for example, but 1,034 deaths were associated with those incidents.

10. Evidently the fact that chemical agents were being used in Laos was not widely known among units of the Lao People's Liberation Army (LPLA). In June 1981, a group of refugees from a village in Vientiane Province reached Thailand and described attacks against them carried out a month earlier by helicopters ''dropping poison'' into their water supply. Lao field units that subsequently entered the village were surprised at the sight of many villagers still suffering from symptoms of acute poisoning. According to the villager, when the Lao military personnel saw the ''small yellow grains'' spread around the village, they were convinced that toxic chemicals had been used on the village and requested medical assistance for those villagers still suffering from nausea and bloody diarrhea.

11. In a 15 December 1981 press conference in Beijing, former Lao Health Ministry Bureau Director Khamsengkeo Sengsthith--who had defected to China--claimed that the Vietnamese were using chemical weapons ''in the air and on the ground'' in Laos, killing ''thousands.'' He asserted that the Vietnamese alone were using such weapons, keeping the matter secret from the Lao. He also stated that 3,000 Soviet advisers are in Laos and ''have taken control'' of the Lao Air Force, while 40,000 to 50,000 Vietnamese troops have reduced Laos to the status of a colony.

12. Obtaining corroborative data for Laos has been difficult simply because of the nature of the fighting there. There have been few major operations. Rather, the reports

6

reflect numerous minor engagements between the opposing forces, and results of these encounters are rarely reported. This is consistent with the observation that the resistance forces are splintered, operating in small, discrete units that emphasize sabotage and unconventional warfare. Finally, in nearly all cases, the chemical use reported has been directed against villages, in the absence of obvious combat operations. This substantiates a Lao pilot's claim that the Vietnamese and Laotian military commands were engaged in a ''H'Mong extermination.'' campaign.

13. Of particular interest are the circumstances surrounding the collection of two physical samples that were found to contain lethal toxins. The first sample was collected after a 13 March 1981 attack on a village between the villages of Muony Chai and Phakhao in the Phou Bia region. In this case, a large two-engine plane reportedly sprayed a mist of a moist, yellow, sticky substance; two villagers and all village animals died. The second sample is from Ban Thonghak, another village in the Phou Bia region. That sample was collected following a 2 April 1981 attack in which an jet aircraft reportedly sprayed a yellow substance; 24 of the 450 villagers died. Seven separate chemical attacks, resulting in 218 deaths, were reported to have occurred in this region in the spring of 1981.

14. It is significant that these attacks took place following a period of escalation in overall resistance activities in the Phou Bia area in the winter of 1980-81. During that period, suppression operations by LPLA and Vietnamese Army (PAVN) forces had achieved only limited success, perhaps spurring both forces on to greater effort. The more intense use of chemical weapons may have been part of this effort.

15. Every qualified interrogator who systematically interviewed the H'Mong refugees concluded that the latter had been subjected to chemical attacks. For example, the US Government medical team returned from Thailand in 1979 convinced that several unidentified chemical warfare agents had produced the symptoms described by the refugees. It was the testimony of a Lao pilot who flew the chemical warfare missions that helped dispel any lingering suspicions that the refugees had fabricated or embellished the stories. His detailed description of the Lao, Vietnamese, and Soviet program to defeat the H'Mong resistance with chemical agents appears in annex B.

7

16. The Lao pilot described the chemical rocket he had fired as having a more loosely fitting warhead than a conventional rocket. In 1977 a H'Mong resistance leader found a US 2.75-inch rocket with a modified Soviet warhead that fits this description. In further corroboration, other sources reported that US 2.75-inch rockets were fitted with lethal chemical warheads by Soviet and Vietnamese technicians at facilities in three Laotian provinces. Munitions storage facilities suitable for storing chemical agents and weapons have been identified in each of these provinces. The aircraft types-L-19s, T-41s, T-28s, and AN-2s--most often reported by the H'Mong refugees as being used to deliver chemical agents have been identified as based on airfields in northern Laos throughout this period.

Kampuchea

G.

17. For Kampuchea we have reports of 124 separate attacks, from 1978 to the present, in which lethal chemicals caused the deaths of 1,014 individuals. Here again, the mortality figure represents a minimum because some reports state only that there were deaths and do not provide a number. The earliest reports cite attacks in Ratanakiri Province, in the northeastern corner of the country (see map, figure 2). Reports from 1979 to the present show the use of lethal chemicals primarily in the provinces bordering on Thailand. The greatest use of chemical agents apparently has been in Battambang Province (51 reported incidents); Pursat Province has suffered the next highest frequency, with 25 reported incidents. These numbers are consistent with the overall high level of military activity reported in the border provinces.

18. A review of reports from all sources provides specific support for 28 of 124 reported attacks. There is, in addition, some circumstantial evidence that in all reported instances some form of attack took place. This evidence includes reports of troop movements, supply transfers, operational plans, postoperation reporting, and air activity reports. It indicates that military activity took place at the time and place of every incident reported to involve lethal chemical agents. In some cases, it provides strong circumstantial indications that the action involved chemical substances--for example, the movement of chemicals and personal protection equipment in the area.

8

1

19. US analysis of contaminated vegetation samples from a March 1981 attack showed high levels of three trichothecene toxins in a combination that we would not expect to be found in a natural outbreak in this environment. These three trichothecenes at the levels found on the vegetation would produce the vomiting, skin irritation and itching, and bleeding symptoms. Water samples taken from the area of the same attack also contained trichothecene toxins. Control samples from nearby areas confirmed that these toxins are not indigenous to the locale. (Details on the sample analysis appear in annex D.)

There is also ample evidence of military activ-20. ity at the place and time of the acquisition of the samples. PAVN defectors described plans for multiregimental sweep operations to be conducted along the border in north-western Battambang Province before the end of the dry season in May. Actual fighting, however, continued to be characterized by guerrilla tactics on both sides, including, according to one PAVN defector, ''staging ambushes, laying minefields, and use of deception.'' Indeed, DK forces were ordered to avoid largescale operations, to limit combat operations to scattered sapper attacks. Such information is consistent with other reports of PAVN forces spreading toxic chemicals along streams and roadsides and around villages, and firing toxic gas shells against enemy positions. In sum, the Phnom Melai sector (in which Phnom Mak Hoeun is located) was described as an ''anthill of DK activity, '' and actions reported during March were ''sporadic firefights'' around Phnom Mak Hoeun involving PAVN forces.

21. In Kampuchea as in Laos, the period of late 1980 through spring 1981 was one of intensified Vietnamese operations to suppress the resistance, and the Vietnamese may have considered the use of toxins an effective means of breaking the will of the opposing forces.

22. Additional supporting evidence was derived from blood samples drawn from victims of PAVN chemical use that reportedly occurred on 19 September 1981 in the Takong area. (Blood analysis appears in annex E.) Takong is in the same general area as Phnom Mak Hoeun--that is, the central region of the Battambang Province - Thailand border. Again, there is no independent confirmation of the accounts of the attacks, but medical personnel visiting the DK field hospital examined the victims and obtained blood samples. Analysis of these samples suggested the use of tricothecenes.

9

23. The chemicals used in the 19 September Takong attack were dispersed (according to the DK soldiers affected) as a gas or powder, and as a poison to water. The gas or powder was released from containers by tripwires in the area of the rear forces. This description is consistent with the other reporting in this area and time.

24. In sum, substantial evidence on the Vietnamese use of chemical weapons existed before the discovery of trichothecene toxins in vegetation and water samples. We share the Thai's concerns about chemical attacks against their own people, especially after one Thai died and others became ill from Vietnamese poisoning. In May 1981 the Thai captured two Vietnamese in the act of poisoning water with cyanide in a Thai relocation camp. A number of reliable reports indicate that it is common practice for PAVN units to poison water and food used by the DK forces.

Afghanistan

25. Attacks with chemical weapons against the Mujahedin guerrillas in Afghanistan were reported as early as six months before the Soviet invasion on 27 December 1979. The reports specify only that Soviet-made aircraft were used to drop chemical bombs, with no clear identification of Soviet or Afghan pilots, or of the specific agents used. A number of Afghan military defectors stated that the Soviets provided the Afghan military with chemical warfare training as well as supplies of lethal and incapacitating agents.

26. For the period from the summer of 1979 to the present, we have reports of 43 separate chemical attacks with more than 3,000 chemical-associated deaths (see map, figure 3). Ten separate chemical attacks, killing considerable numbers of persons, were reported in the first three months of 1980. All of the reports came from northeastern Afghanistan and provide the highest percentage of reported deaths. By the spring and summer of 1980, chemical attacks were reported to have occurred in all areas of concentrated resistance activity. Reports of chemical weapon use in 1981 essentially parallel 1980 reporting with respect to frequency and location of attack. Of the 43 chemical attack reports, 36 have come from human sources, including Afghan Army deserters, Mujahedin resistance fighters, journalists, and US physicians. For 24 of the reported 43 attacks we have additional independent evidence supporting allegations of chemical attacks. In seven instances we have additional

5

human reporting. For example, an Afghan insurgent provided an eyewitness account of a 6 July 1980 attack on a village 10 kilometers east of Darae Jelga in Vardak Province. The insurgent reported that a Soviet MI-24 helicopter gunship dropped a bomb that, upon explosion, released a lethal chemical. A separate report from a reliable source confirmed that Soviet aerial bombing attacks were taking place during this period on villages in Vardak (as well as Lowgar and Parvan Provinces.)

27. Support for 20 of the reported incidents comes from information on Soviet or Afghan Army combat operations that were in progress in areas at times approximating those of a reported chemical attack. In a few cases, reporting is quite specific. The following sequence, for example, occurred in a small valley in Qandahar Province in early June 1981: Soviet combat groups engaged rebel forces in that valley during a two-week period, according to an Afghan exile. The situation worsened for the Soviets and an airstrike was conducted. The Afghan exile reports that a Soviet helicopter delivered a single rocket, which released a chemical that killed 16 insurgents. Nearly all reports state that chemicals were delivered by aircraft or helicopters, with a few reports describing chemical artillery rounds.

28. Many reports from different sources strongly support the use of irritants to drive the insurgents into the open to expose them to attack with conventional weapons, and incapacitants to render them tractable for disarming and capture. On several occasions in April 1980, for example, Soviet helicopter pilots dropped ''gas bombs'' on insurgents, evidently to drive them from caves.

29. Victims of Soviet attacks, conducted to flush them from caves, describe symptoms that cannot be associated with riot control agents like CN and CS or even Adamsite. Medical examinations of some of the victims include reports of paralysis, other neurological effects, blisters, bleeding, and sometimes death. While none of the agents being used in Afghanistan have been positively identified through sample analysis, it seems clear that the agents being used are far more toxic than the irritants cited above.

30. Afghan military defectors have provided lists of the Soviet agents stockpiled in Afghanistan and described where and when some of them have been used. The list

11

Ŧ

included nerve agents, phosgene, phosgene oxime, sulfur mustard, nitrogen mustard, and lewisite. The agents used, plus the time and location of the attacks, generally correspond to the refugee reports and recorded military operations. Afghan military defectors have also pinpointed where they are stored.

31. Soviet operational personnel decontamination stations were observed at two locations in Afghanistan and a chemical decontamination field unit was deployed during a sweep operation of the Konar Valley in 1980. In addition, Soviet personnel have been observed wearing chemical protective equipment. At Shindan, TMS-65 decontamination units were deployed in a classical operational mode. This suggests that the chemical battalions have performed an operational role in Afghanistan connected with offensive chemical use. A Soviet chemical specialist told an American news correspondent that his mission was to examine villages after a chemical attack to determine whether it was safe to enter or required decontamination. An Afghan pathologist who defected described how he accompanied Soviet chemical warfare personnel into contaminated areas to collect soil, vegetation, and water samples after Soviet chemical attacks. The Soviets, according to firsthand experience of former Soviet chemical personnel, do not require decontamination equipment in an area where chemical bombs are stored or loaded on aircraft. We thus associated the deployment of this equipment in Afghanistan with the active employment of casualty-producing chemical agents.

32. In sum, the eyewitness testimony of Afghan refugees and journalists about chemical warfare activities is supported by defectors, as was the case in Laos and Kampuchea. Other evidence supports the judgment that chemical agents have been used and that Afghan and Soviet military operations took place in almost every area where we have reports of chemical attacks.

What Chemical Agents Are Being Used?

33. The specific chemical agents being used in Laos, Kampuchea, and Afghanistan cannot be determined without collection and analysis of at least one of the following: environmental samples contaminated with agent, the munitions used to deliver agents, or biological specimens from victims of an attack. A study by medical-toxicological experts of symptoms exhibited by individuals exposed to toxic agents does provide a good indication of the general

12

3

class of chemical agent used. Thus, the range of clinical manifestations from chemical agents as reported by a US Army investigative team resulted in the determination that nerve agents, irritants such as CS, and a highly toxic hemorrhaging chemical or mixture of chemicals were used in Other medical-toxicological personnel arrived at the Laos. same determination and further indicated that toxins such as the trichothecenes were a probable cause of the lethal hemorrhaging effect seen in Kampuchea as well as Laos. Symptoms reported by the DK in Kampuchea and the Mujahedin in Afghanistan were in many cases similar to those reported by the H'Mong in Laos. In addition, symptoms reported from Afghanistan and Kampuchea indicated that a highly potent, rapid-acting incapacitant ''knockout'' chemical also was being used. Mujahedin victims and witnesses to chemical attacks reported other unusual symptoms, including a blackening of the skin, severe skin irritation with multiple small blisters and severe itching, severe eye irritation, and difficulty in breathing--suggesting that phosgene oxime or a similar substance was used.

Collecting samples possibly contaminated with a toxic 34. agent during or after a chemical assault is difficult under all circumstances but particularly when the assault is against ill-prepared people without gas masks and other protective equipment. Obtaining contaminated samples that will yield positive traces of specific chemical agents is dependent on a number of factors. These include the persistency of the chemical, the ambient temperature, rainfall, wind conditions, the media on which the chemical was deposited, and the time, care, and packaging of the sample from collection to analysis in a laboratory. Many standard chemical warfare agents are nonpersistent and disappear from the environment within a few minutes to several hours after being dispersed. These include, for example, the nerve agents Sarin and Tabun; the blood agents hydrogen cyanide and cyanogen chloride; the choking agents phosgene and diphosgene; and the irritant phosgene oxime. Other standard CW agents--such as the nerve agents VX and thickened Soman and the blistering agents sulfur mustard, nitrogen mustard, and lewisite--may persist for several days to weeks depending on weather conditions. The trichothecene toxins have good persistency but may be diluted to below detectable concentrations by adverse weather conditions. To maximize the chances of detection, sample collections should be made as rapidly after a chemical assault as possible, and with many agents this means minutes

13

to hours. Under the circumstances of Southeast Asia and Afghanistan this has simply not been possible. While numerous samples were collected, few of them held any realistic prospect of yielding positive results. It is fortunate that trichothecenes are sufficiently persistent to allow detection several months after the attack.

35. Samples have been collected from Southeast Asia since mid-1979 and from Afghanistan since May 1980. To date about 50 individual samples--of greatly varying types and usefulness for analytical purposes -- have been collected and analyzed for the presence of traditional CW agents, none of which have been detected. On the basis of recommendations by medical and toxicological experts and of findings by the CSL, many of the samples have been analyzed for the trichothecene group of mycotoxins. Four samples, two from Kampuchea and two from Laos, were found to contain high levels of trichothecene toxins. Preliminary results of the analysis of blood samples drawn from victims of an attack indicate the presence of trichothecene (T-2) metabolite, but quantification of their levels is pending.

36. The accompanying table lists the chemicals and their probability of use in Afghanistan by Soviet and, in Southeast Asia, by Vietnamese and Laotian forces. The judgments shown in this table are based on sample analysis, on collateral and special intelligence, and on medical and toxicological evaluations.

Soviet Chemical Warfare Activities

37. Evidence accumulated since World War II clearly shows that the Soviets have been extensively involved in preparations for large-scale offensive and defensive chemical warfare. We have identified the chemical warfare agents and delivery systems they have developed, probable production and storage areas within the USSR, and continuing research, development, and testing activities at the major Soviet chemical proving grounds. None of the evidence indicates any abatement in this program. The Soviets have shown a strong interest in improving or enhancing their standard agents for greater reliability and effect. Their large chemical and biological research and development effort has led them to investigate other kinds of CW agents, particularly the toxins. A bibliography on Soviet research in the toxin field is included in annex D.

14

UNCLASSIFIED

1

Degrees of Confidence in Identification of Specific Classes of Chemicals Used in Southeast Asia and Afghanistan^a

	Laos	Kampuchea	<u>Afghanistan</u>
Trichothecene toxins	Confirmed	Confirmed	Suspected
Nerve agents	Probable	Probable	Probable
Irritants	Probable	Probable	Probable
Vesicants and urticants	Suspected	Suspected	Probable
Incapacitants	Suspected	Probable	Probable

^a The confidence levels shown refer to the identification of <u>specific</u> chemicals used, <u>not</u> to the probability that some form of lethal chemical was used. We consider the latter a certainty.

15

5

To our knowledge, none of the four countries--38. Vietnam, Laos, Kampuchea, and Afghanistan--has any largescale facility or organization for the manufacture of chemical and biological materials. Nor are they known to have produced even small quantities of chemical warfare agents or munitions. The technical problems of producing large quantities of weapons-grade toxin, however, are not so great as to prevent any of the four countries from learning to manufacture, purify, and weaponize these materials. It is highly unlikely, however, that they could master these functions without acquiring outside technical know-how. The only country known to be providing chemical warfare assistance to these countries is the Soviet Union.

39. The Soviets have had advisers and technicians working in Vietnam, Laos, and Kampuchea for many years, but not until early 1979 did evidence connect the Soviet military directly with chemical warfare activities. The evidence is quite conclusive. For example, in early 1979, Soviet military personnel inspected chemical storage facilities in Paxse. The chemicals inspected were reportedly those that cause ''stomach sickness and death.'' This important piece of evidence was supported by several reliable reports that provided more detail on the mid-February visit and on another Soviet inspection by chemical warfare experts in June 1979.

40. Another reliable report stated that the chemical section in one Lao province prepared Soviet-manufactured chemical items for inspection by a Soviet military team in early 1979. A seven-man team of Soviet chemical artillery experts, accompanied by Laotian chemical officers, inspected chemical supplies and artillery rounds at the Xeno storage facility in mid-1979. One report stated that the Soviet team would be inspecting the same chemical explosives used to suppress the H'Mong resistance in the Phou Bia area.

16

UNCLASSIFIED

CONCLUSIONS

Laos. We conclude from all the evidence that selected Lao and Vietnamese forces, under direct Soviet supervision, have employed lethal trichothecene toxins and other combinations of chemical agents against the H'Mong resistance forces, including their villages, since at least 1976. Thousands have died, have been severely injured, or were driven from their homeland by the use of these agents.

Kampuchea. The evidence strongly supports the conclusion that the Vietnamese have similarly been using lethal trichothecene toxins and other combinations of chemical warfare agents on Democratic Kampuchean forces and other resistance groups since at least 1978.

Afghanistan. We conclude that Soviet forces in Afghanistan have used a wide variety of lethal and nonlethal chemical agents on Mujahedin resistance forces and Afghan villages since the Soviet invasion in December 1979. Afghan Government forces probably used chemical weapons before the Soviet invasion, but we cannot identify the types of agents used. It has not been possible to identify the agents used by the Soviets through sample analysis, but a number of Afghan military defectors have named the agents brought into the country and have described where and when they were used. That information has been correlated with all other evidence, including the reported symptoms. We conclude that nerve agents, phosgene oxime, and various incapacitants, and irritants have been used. Other agents and toxic smokes are also available in country, but we cannot state confidently that they have been used. Some of the reported symptoms are consistent with those produced by lethal or sublethal doses of trichothecene toxins, but our evidence is not conclusive.

The Soviet Role. We conclude that the Soviets either provided the toxin weapons directly or provided the toxins for weaponization in Vietnam and Laos. A common practice in the Soviets' own military forces is to store agents in bulk and move them to the field for munitions fill as needed. Our assumption that this practice is also followed in Indochina and Afghanistan is supported by a number of reports, which specify that Soviet technicians supervise the shipment, storage, filling, and loading on aircraft of the chemical munitions. The dissemination techniques reported

17

and observed are evidently drawn from years of Soviet chemical warfare testing and experimentation. No intelligence is at hand to support any alternative explanation, but we cannot completely rule out the possibility that Soviet technical assistance has enabled at least the Vietnamese to conduct an indigenous toxin production program.

Motivation for Chemical Weapons Use. In the course of the analysis, we have posed the question: ''Is there a military-strategic or tactical rationale for the systematic use of chemical weapons in Laos, Kampuchea, and Afghanistan?'' We conclude that the military problems faced in all three countries--as viewed from the perspective of the Soviets and their allies--make the use of chemical weapons a militarily effective way of breaking the will and resistance of stubborn guerrilla forces operating from relatively inaccessible protected sanctuaries. These weapons offer substantial advantages over conventional weapons. In all three countries the resistance was able through conventional means to frustrate Soviet and client-state objectives of extending and consolidating control over the countries attacked. The Soviets probably reasoned that attainment of these objectives -- as quickly and cheaply as possible -- justified use of chemical weapons and outweighed a small risk of exposure and international condemnation. They may well have calculated that they and their allies could successfully deny or counter charges that chemical weapons had been used, recognizing that it would be most difficult to compile incontrovertible evidence from inassessible areas of Southeast Asia and Afghanistan. In addition, the Soviet military very likely consider these remote areas as providing unique opportunities for the operational testing and evaluation of chemical weapons under various tactical conditions.

We found support for this conclusion from Third World officers who had attended the Soviet Military Academy of Chemical Defense in Moscow. According to their Soviet instructor, three types of chemical agents may be used during the ''initial stages'' of local wars: ''harassing agents (CS, CN, DM), incapacitants such as psychochemicals (BZ) or intertoxins [sic (possibly enterotoxins)], and herbicides.'' During the ''decisive phase, lethal agents can be employed under certain circumstances.'' In a ''local war, chemical weapons can be used to spoil enemy efforts to initiate operations, even if the enemy has not used them first.'' The foreign officers' accounts, including detailed descriptions of the Soviet chemical warfare program, supports the conclusion that the Soviets consider chemical weapons an effective and acceptable means of warfare in local conflicts.

18

UNCLASSIFIED

Annex A

<u>Tabulations of Reported Chemical Warfare</u> <u>Attacks in Laos, Kampuchea, and Afghanistan</u>

This annex comprises three tables summarizing chronologically, by location, number, and associated deaths, the chemical attacks reported to have occurred in Laos, Kampuchea, and Afghanistan between 1975 and 1981. The tables were compiled from a large volume of intelligence reports on such attacks. Every effort was made to correlate individual allegations with collateral information and to eliminate double counting. The number of fatalities shown almost certainly fall short of actual totals, because our coverage is inevitably incomplete, and many reports failed to provide casualty numbers.



UNCLASSIFIED

1

Table A-1

Laos: Summary of Reported Chemical Attacks and Associated Deaths, 1975-81

<u>Time Period</u>	Area	Attacks ^a	${\tt Deaths}^{\tt b}$
Summer 1975	Vientiane	2	25+
Fall 1976	Phou Bia Savannakhet	8 1	10 10
Winter 1976-77	Phou Bia	2	16
Spring 1977	Phou Bia Khammouan	6 2	66+ 1
Summer 1977	Phou Bia	6	95
Fall 1977	Phou Bia	1	25
Winter 1977-78	Phou Bia Savannakhet	10 6	1,328+ 224
Spring 1978	Phou Bia	34	969+
Summer 1978	Phou Bia	22	664+
Fall 1978	Phou Bia	19	572
Winter 1978-79	Phou Bia	5	15+
Spring 1979	Phou Bia	36	257+
Summer 1979	Phou Bia	5	239+
Fall 1979	Phou Bia Xaignabouri	10 2	56 24+
Winter 1979-80	Phou Bia	4	10+
Spring 1980	Phou Bia	3	24
Summer 1980	Phou Bia	6	187+

A-2 UNCLASSIFIED

Approved for Release: 2015/01/05 C05184036

UNCLASSIFIED

1

Table A-1 (continued)

Time Period	Area	<u>Attacks^a</u>	$\underline{\mathtt{Deaths}}^{\mathtt{b}}$
Fall 1980	Xaignabouri	1	12
	Phou Bia	7	88+
	Savannakhet	3	1+
Winter 1980-81	Xaiqnabouri	2	57
	Phou Bia	4	82
	Vientiane (SE)	1	1+
Spring 1981	Houaphan	2	?
	Phou [¯] Bia	7	218
	Vientiane (S)	1	
Summer 1981	Phou Bia	1	?
Fall 1981	Phou Bia	4	500+
	Khammouan	3	534+
		226	6.310 +

^a This tabulation omits 35 attacks, accounting for 194 deaths, that were not located in the reports. The totals overall were 261 attacks and more than 6,504 deaths.

^b A plus sign indicates that the report(s) of deaths gave a minimum figure. In some cases (shown with a question mark) deaths were reported, but no number was given. Other reports (signified with a dash) gave no information on fatalities.

> A-3 UNCLASSIFIED

.....

A,

UNCLASSIFIED

Table A-2

Kampuchea: Summary of Reported Chemical Attacks and Associated Deaths, 1978-81

Time Period	Area	<u>Attacks</u>	Deaths ^a
1978	Ratanakiri	5	?
Summer 1979	Kampong Speu	4	37
Fall 1979	Siem Reap	1	
	Battambang	4	22+
	Pursat	2	1+
	Koh Kong	2	6+
	Kampot	1	3
	Kampong Chhnang	2	118
Winter 1979-80	Battambang	12	64+
	Pursat	5	21+
	Koh Kong	2	4
Spring 1980	Battambang	3	20+
	Pursat	8	24+
	Koh Kong	5	13
Summer 1980	Siem Reap	1	82+
	Battambang	3	23+
	Pursat	2	7
	Koh Kong	3	
Winter 1980-81	Battambang	8	
	Pursat	2	3
Spring 1981	Preah Vihear	1	
	Battambang	12	163+
	Pursat	3	42+
	Koh Kong	1	
	Kampot	1	
Summer 1981	Battambang	3	7+
	Kampong Thom/Cham	1	

A-4 UNCLASSIFIED

Approved for Release: 2015/01/05 C05184036

UNCLASSIFIED

Table A-2 (continued)

Time Period

Fall 1981

儶

Area	Attacks	Deaths [~]
Siem Reap	16	305
Battambang	6	16
Pursat	3	 , "
Koh Kong	1	-
Kampot	1	`
	124	981

а

^a A plus sign indicates that the report(s) of deaths gave a minimum figure. In some cases (shown with a question mark) deaths were reported, but no number was given. Other reports (signified with a dash) gave no information on fatalities.

4

UNCLASSIFIED

0

Table A-3

Afghanistan: Summary of Reported Chemical Attacks and Associated Deaths, 1979-81

<u>Time Period</u>	Province	<u>Attacks^a</u>	${\tt Deaths}^{\sf b}$
Summer 1979	Badakhshan Parvan Bari ar	1	2,000 8
	Bamian	Ŧ	
Fall 1979	Konarha	1	350
	Farah	1	?
	Herat	1	. ?
٥	Badghisat	ī	· ?
Winter 1979-80	Badakhshan	5	130+
	Takhar	1	*-
۰	Konarha	2	10+
	Nangarhar	1	?
	Bamian	1	?
Spring 1980	Badakshan	1	1+
	Konarha	2	?
	Oruzgan	1	
	Qandahar	1	 ,
Summer 1980	Nangarhar	2	1
	Vardak	1	3
	Herat	2	300+
	Kabul	2	
Fall 1980	Konarha	1	?
	Lowgar	1	4
	Ghazni	1	100
Winter 1980-81	Lowgar	2	?
Spring 1981	Parvan	2	
	Lowgar	3	
	Ghazni	2	?
	Qandahar	1	
Summer 1981	Nangarhar	2	?
	Qandahar	2	16
	Herat	1	119
		47	3,042

A-6 UNCLASSIFIED

Approved for Release: 2015/01/05 C05184036

4

- ^a This tabulation omits some attacks described in the text because they could not be dated or located with high confidence.
- ^b A plus sign indicates that the report(s) of deaths gave a minimum figure. In some cases (shown with a question mark) deaths were reported, but no number was given. Other reports (signified with a dash) gave no information on fatalities.



UNCLASSIFIED

ANNEX B

A LAO PILOT'S STORY

The most complete description of the period 1976-78 came from a Lao pilot who was directly involved in chemical warfare. The pilot, a former LPLA officer who defected in 1979, reported that he flew L-19 and T-41 aircraft equipped to dispense toxic chemical agents on H'Mong villagers in the Phou Bia area of northern Laos. He said that the LPLA, in cooperation with the PAVN, had conducted CW operations in Laos since April or early May 1976. At that time, two LPLA H-34 helicopters conducted a series of shuttle flights transporting rockets to an airfield in Xiangkhoang Province.

Between June and August 1976 the LPLA launched attacks in the area of Bouamlong--in Xiangkhoang Province--that was a stronghold for remnants of the forces of former H'Mong Gen. Vang Pao. The LPLA used L-19 aircraft for rocket attacks in that area aimed at eliminating the H'Mong resistance. LPLA crews responsible for loading rockets on the attack aircraft noted, however, that they were not allowed to use the rockets that had been moved from Long Tieng to Phonsavan, even though Phonsavan was much closer to the Bouamlong target area than Long Tieng, where LPLA aircraft had to rearm. The pilot said that, during nearly three months of flying missions against the Bouamlong area, he flew his L-19 aircraft to Long Tieng to be armed with rockets.

In late 1976 the pilot's L-19 aircraft was rearmed with rockets stored at Phonsavan. Initially, H-34 helicopters were used to transport the rockets from Phonsavan to a depot near the Ban Xon airfield, Vientiane Province, where they were fitted onto racks of the L-19 aircraft for missions in the Phou Bia area. Later, the rockets from Phonsavan were transported to Ban Xon by trucks. All US-manufactured rockets were stored with the tip and canister kept apart; in other words, the two parts had to be joined before being fitted to the racks on the aircraft. The pilot observed, however, that all the rockets transported from Phonsavan to Ban Xon were already assembled.

As part of his routine flight activities, the pilot would check over his aircraft and, in doing so, examine the tip portion of new smoke rockets that had been transported from Phonsavan. He said that most of them appeared ''loose''

B-1

in the portion where the tip and canister joined, whereas the tip and canister of the ordinary explosive-type rockets at Long Tieng were noticeably more tightly connected.

In late 1976, preparation for airstrikes on Kasy (Louangphrabang Province) and in new areas of Phou Bia, the pilot said he began carrying two or three PAVN staff officers, sometimes accompanied by an LPLA staff officer, in T-41 aircraft for reconnaissance over the target areas. When these airstrikes were launched, the defector pilot initially flew his L-19 aircraft on missions with another pilot and an LPLA staff officer sitting in the rear seat. After two or three weeks, however, PAVN staff officers, who spoke excellent Lao, began alternating in the rear-seat role with the LPLA officers. Before each mission, the PAVN or LPLA staff officer would go over target areas outlined on situation maps--which then were taken along--and would point out the targets to be attacked. The source noted that at no time did the PAVN staff officer sitting in the back seat of his aircraft communicate with LPLA officers on the ground, as did the LPLA staff officers. A new PAVN officer was used on each mission always assigned for each of the T-41 reconnaissance and L-19 airstrike missions in the H'Mong areas. The average age of these PAVN staff officers was midfourties.

The source related that before flying L-19 airstrike missions with a full load of rockets he was often warned by an LPLA commander to fly at above-normal altitudes when firing rockets to preclude hazard to the occupants of the aircraft. For this reason the pilot surmised that the ''smoke'' rockets fired at the H'Mong people were unusual. He was able to observe that the ''smoke'' rockets detonated in the air and that some produced white smoke with a mixture of blue, while others produced red smoke with a mixture of yellow. The ordinary, explosive-type rockets detonated on impact. The commander or his designated representative told the pilot before every mission that the operations--called Extinct Destruction Operations--were intended to ''wipe out'' the reactionary H'Mong people.

Before a mission involving ''smoke rockets,'' the commander warned the pilots to keep the operation secret. The source said that, during the nearly two years in which he flew rocket missions, he learned from the LPLA staff officers accompanying him that there were two types of rockets. The first, mostly ''smoke'' rockets, were to be fired at targets far away from LPLA and PAVN troops to avoid exposing them

B-2

UNCLASSIFIED

to the poison smoke. The second was of the ordinary explosive type, considered a ''close support'' rocket that could be fired near LPLA and/or PAVN troop positions. Initially, the L-19 aircraft carried eight rockets--five ''close support'' and three ''smoke'' rockets. Later, only four rockets, mainly of the ''smoke'' type, were carried.

After each mission in which chemical warfare rockets were used, the pilot was returned to a ''rest house'' at Phonsavan, where an LPLA doctor and nurse would examine him. He said that after his missions, especially in 1978, he was particularly well treated by the examining doctor and watched very closely by the LPLA nurse. Those L-19 aircraft pilots assigned to missions utilizing chemical warfare rockets had special privileges, including additional flight pay and free meals at the Phonsavan cafeteria. In October 1978 the LPLA stopped using L-19 aircraft on combat missions and began using Soviet MIG-21s for chemical attacks on the Phou Bia areas.

Corroborating Evidence. Several H'Mong reports provide significant substantiation of the testimony of the Laotian pilot. The chief of eight villages, for example, described attacks covering all seven days of the week of 5 June 1976 in the Bouamlong area, Xiangkhoang Province. He described L-19 aircraft firing rockets that produced red and green smoke. Ten villagers were killed by gas and 30 by shrapnel. Most of the H'Mong reports documented by a US foreign service officer in June 1979 and a Department of Defense medical team in October 1979 are consistent with the Laotian pilot's testimony. H'Mong observers familiar with military aircraft reported L-19s until late 1978. After that time, reports described jets or ''MIGs'' and some accurately described Soviet AN-2s.

A review of information back to 1975 shows L-19 and T-28 or aircraft were operating from airfields in northern Laos--including the one at Phonsavan, where AN-2s were seen in 1978. Our failure to observe chemical decontamination equipment at the airfields, moreover, does not rule out the handling of chemical munitions. The Soviets supervise the chemical warfare activities in Laos; we assume, therefore, that chemical munitions are handled in about the same manner as in the USSR. No protective clothing or special decontamination equipment is required for loading chemical bombs on aircraft and helicopters at chemical munitions test ranges, according to former Soviet chemical warfare personnel.

in

B-3

The Laotian pilot's description of the rockets used on the L-19 was corroborated by other sources. An H'Mong refugee, a former commander of a 500-man resistant force, reported that in 1977 he found a rocket canister and a separated warhead that he believed were the kinds used by the Vietnamese and Laotians. The canister, he said, had authentic US markings identifying it as a US-manufactured 2.75-inch rocket, as well as reportedly three lines of Russian writing (which he could not translate). Another H'Mong source, who reportedly had been trained as a liaison officer and ordnance expert before the Communist takeover of Laos in 1975, stated that he too believed the rocket canister was of US manufacture and that the Soviet technicians in Laos had modified the upper stage to contain a poisonous (lethal) chemical.

The diameter of the warhead was reported to be 12.5 centimeters (5 inches), probably a measurement taken on a modified warhead because the United States does not have a 5-inch warhead for the 2.75-inch ''rocket motor.'' During the Vietnam conflict, about 35 million US-manufactured, conventional 2.75-inch rockets were sent to the war zone, and many tens of thousands of these no doubt fell into North Vietnamese hands when South Vietnamese forces collapsed. The Vietnamese may be using some of these rockets with existing loads, but modified warheads for the 2.75-inch rocket motor could easily be fabricated in Vietnam and filled with a lethal or nonlethal agent in Laos, especially with Soviet assistance. According to US experts, fabrication of a warhead 5 inches in diameter, necked down to fit the 2.75-inch rocket, could be accomplished by trained technicians in a small, well-equipped machine shop and laboratory.

B-4

UNCLASSIFIED

Annex C

Final Report of DASG Investigative Team: Use of Chemical Agents Against the H'Mong in Laos

Authors:

Charles W. Lewis, M.D., COL, MC Chief, Dermatology Service, Brooke Army Medical Center, Fort Sam Houston, Texas

Frederick R. Sidell, K.D., Chief, Clinical Resources Group, US Army Biomedical Laboratory, Aberdeen Proving Ground, MD. 21010

William D. Tigertt, M.D. (Brigadier General, RET, USA) Professor of Pathology, University of Maryland, Baltimore, Maryland

Charles D. Lane, LTC, Southeast Asia Desk Officer, OACSI, Department of the Army, Washington, D.C.

Burton L. Kelley, SP5, USA, Dermatology Technician, Brooke Army Medical Center, Fort Sam Houston, Texas

C-1

UNCLASSIFIED

Approved for Release: 2015/01/05 C05184036

ŝ

From 28 September to 12 October 1979 a team from the office of The Surgeon General was in Thailand to investigate allegations of the use of chemical agents against H'Mong tribesmen in Laos.

The team visited the following H'Mong refugee camps of northern Thailand: the detention center at Nhong Khai, the large H'Mong camp at Ban Vanai, and two smaller camps at Nam Yao and Mae Charmin. As the great majority of refugees as well as the H'Mong leadership are at Ban Vanai most of the interviews were obtained there.

Entrance and exit briefings concerning the team's mission were held at the US Army Biomedical Laboratory, Aberdeen Proving Ground; the State Department, Washington, D.C.; the U.S. Embassy, Bangkok; the Thailand Army Surgeon General; refugee camp officials, as well as the U.S. Army Surgeon General and Assistant Chief of Staff for Intelligence.

The team was prepared to obtain blood and skin samples (for cholinesterase activity and study of pathological changes respectively) from those exposed to chemical agents. For such samples to yield meaningful results they must be taken within 6-8 weeks of exposure. Since the last reported exposure was in May 1979 no samples were collected.

Interviews were conducted through interpreters; one interpreter was an employee of the U.S. Consulate at Udorn, and the remainder were hired from among the refugees. The interpreters screened those refugees who volunteered to talk to the team and selected only those who had or been eyewitnesses to or had themselves been exposed to an agent attack. Team members interviewed 40 men, 2 women, and a 12 year old girl. Each interview took 1-2 hours. To achieve conformity a prepared questionnaire was used as a guide.

The chemical attacks reportedly occurred between June 1976 and May 1979 (table C-1). The absence of reports of attacks after May 1979 may be because very few refugees crossed the Mekong River after that time because of heavy rains and flooding from June to September 1979. Most of the early reports were of the use of rockets releasing the agent, but beginning in the fall of 1978 the majority of the attacks were carried out by aircraft spraying a yellowish substance which ''fell like rain.'' The sites of the attacks, which were concentrated around the H'Mong stronghold in the mountainous Phou Bia area, are also listed in table C-1.

C-2

The team was given a plastic vial containing pieces of bark stained by a yellow substance which several H'Mong refugees claimed was residue from an aircraft spray attack in April 1979. Preliminary chemical analysis of the sample indicates that no standard chemical agent is present, i.e., an agent listed in TH 8-285 (U.S. Army, May 1974). A complete report of this analysis will be submitted upon completion of further studies.

A similar series of interviews was conducted by State Department Officials in June 1979. From the signs/symptoms described and observed the following is suggested:

1. At least two, and possibly three, different chemical agents may have been used, such as:

(a) A nerve agent (five or six individuals reported symptoms that could be attributed to a nerve agent).

(b) An irritant or riot control agent (1/3 of the interviews).

(c) Over half of the interviews indicated such a variety of signs and symptoms that it is difficult to attribute them to a single known agent.

2. It is possible that in some cases, two or more agents were combined.

(a) Reported signs and symptoms suggesting a nerve agent include sweating, tearing, excessive salivation, difficulty in breathing, shortness of breath, nausea and vomiting, dizziness, weakness, convulsions, and death occurring shortly after exposure.

(b) Reported signs and symptoms suggesting a riot control or irritant agent include marked irritation or burning of the eyes with tearing and pain; irritation and burning of the nose and throat; coughing, burning and tightness in the chest; headache; and nausea and vomiting in a few cases.

(c) Reported signs and symptoms not related to any known single agent include a mixture of the above plus the features of profuse bleeding from mucous membranes of the nose, lungs, and gastrointestinal tract

C-3

UNCLASSIFIED

Approved for Release: 2015/01/05 C05184036

UNCLASSIFIED

with rapid death of the affected individuals in some instances. Many of these effects were similar to those described in attacks during the war in Yemen.*

Estimates from the H'Mong interviewed indicate that approximately 700-1,000 persons may have died as a result of the use of chemical agents, and that many times this number were made ill. It was reported that on numerous occasions entire villages were devastated by these agents leaving no survivors.

In the episodes described most of the animals exposed to the chemical agents were killed. Generally, all chickens, dogs, and pigs died, and to a lesser extent, the cattle and buffalo.

On several occasions it was reported that where these agents settled on tree and plant leaves, many small holes appeared in the leaves within two or three days. Rarely did agent exposure result in the defoliation or death of the plants.

CONCLUSIONS:

The conclusions of the team based upon interviews obtained from H'Mong refugees are as follows:

1. Chemical agents have been used against the H'Mong.

2. The reported effects of these agents suggest the use of a nerve agent, a riot control agent, and an unidentified combination or compound.

* SIPRI The Problem of Chemical and Biological Warfare, Volume 5, The Prevention of CBW, page 255, Humanities Press, Inc., 300 Park Avenue South, New York, N.Y. 10010

C-4

UNCLASSIFIED

Approved for Release: 2015/01/05 C05184036

UNCLASSIFIED

Table C-1

ñ

\$

REPORTS OF PROBABLE CHEMICAL AGENT ATTACKS

DATE	LOCATION	METHOD OF ATTACK	MATERIAL USED (SMOKE OR GAS)
Jun 76	Pou Mat Sao	Plane, rockets	Red and green
Jan 77-	Pha Khao	Plane, rockets	Yellow, red. green
Oct 78		,	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,
Mar 77	Nam Theuna	Plane, rockets	Red and vellow
Apr 77	Houi Kam Lang	Plane, rockets	Yellow
May 77	Pha Khae	Plane, rockets	Red
May 77	Nam Moh	Plane, rockets	Yellow
May 77	Pha Ngune	Bi-plane spraying	Yellow gas
-	5	Plane, rockets	Yellow
1977 - 78	Phu Seu	Plane, rockets	Red, green, vellow
x 3			·····
Jan 78	Houi Xang	Plane, rockets	Red and green
Feb 78	Sane Mak Ku	Plane, rockets	Yellow
Feb 78	Tham Se Sam	Plane, rockets	Yellow and black
	Lein		
Feb 78	Kio Ma Nang	Plane, rockets	Yellow
Mar 78	Mouong Ao	Plane, rockets	White
Mar 78	Khieu Manang	Plane, rockets	Green
Apr 78	Tha Se	Plane, rockets	Yellow
Jun 78	Pha Phay	Plane, rockets	Yellow
Jun 78	Phou Seng	Plane, rockets	Red, white, black
Jul 78	Phou Bia	Plane, rockets	Red
Jul 78	Ban Nam Mo	Plane - spray	Yellow
Jul 78	Phou Lap	Plane - rockets	Yellow
Aug 78	Pha Houai	Plane - rockets	Red and green
Aug 78	Ban Thin On	Plane - rockets	Green and red
Aug 78	Bouam Long	Plane - rockets	Red, green, yellow
Sep 78	Pha Koug	Plane - rockets	Yellow
Sep 78	Ban Nam Tia	Plane - spray/	Yellow, green, red
	•	rockets	, , ,
Sep 78	Pha Na Khum	Plane - rockets	Red
Oct 78	Phou Bia	Plane - rockets	
Oct 78	Ban Done	Plane - spray	Yellow
Oct 78	Phou Bia	Plane - rockets	White, green, red
Nov 78	Phou Chia	Plane - rockets	White, red
Feb 79	Pha Mat	Plane - spray	Yellow
Feb 79	Tong Moei	Plane - rockets	Yellow and red
Mar 79	Pha Mai	Plane - spray	Yellow
Apr-May	Pha Mai	Plane - spray	Gray-white
79 x 4			
Mar-May	Pha Mai	Plane - spray	Yellow
79 x 6			
May 79 ·	Phou Chia	Plane - spray	Yellow
May 79	Moung Phong	Plane - rockets	Red
		C-5	•
UNCLASSIFIED

ł

3

Table C-1 (continued)

Department of State Interviews

DATE		LOCATION	METHOD OF ATTACK	MATERIAL USED (SMOKE/GAS)
Oct 71	7	Phu Hay, S of Phu Bia	Plane (L-19) rockets	Yellow-grey gas
Unk 78	8	Pa Sieng, S of Phu Bia	Plane unk bomb	Yellow cloud
Feb 78	8	Ban Nam Luk, S of Phu Bia	Planes (L-19) sprav (?)	Yellow/white gas
Feb 78	8	20 Kms SE of Phu Bia	Plane unk spray (?)	Yellow, provided sample
Feb 78	8	Ban Ko Maí	Plane unk bomb	Yellow
Mar 78	8.	Pha Houei	Plane (MIG?) sacks, burst in air	Brown gas
Mar 78	8	Ban Na Pong	Plane (jet?) not described	Yellow
Apr 78	8	Ban Phamsi	Plane not de- scribed	White, green, blood colored
May-A _l 78	pr	Ban Nong Po	Plane (MIG?) cloud	Yellow-brown like rain
Jun 78	8	Ban Nam Teng	<pre>Plane unk rocket(?)</pre>	Yellow gas
Jun 78 May 79	8- 9	Ban Don area	Plane (jet?) spray	Yellow rain
Mid-78	8	1-3 kms NE of	Plane unk rocket	Red gas
		Phu Bia	burst in air	
Oct 78	8	Nam Kham	Plane (L-19) rockets, air burst	Yellow cloud
Oct 78	8	6 kms N of Phou Khao	Plane (L-19?) rockets, air burst	Red cloud
Oct 78	8	3-4 kms N of Phu Bia	Plane (L-19?) rockets, air burst	Yellow gray fog
Nov 78	8	Phou Xang Noi	Plane (MIG?) spray	Yellow and blue cloud
Nov 78	8	nr Phu Bia	Plane unk bomb, air burst	Yellow substance
Nov 78	8	NE of Pha Khao	Plane (L-19) rocket, air burst	Yellow gas
Apr 79	9	Ban Nouia Pong	Plane (MIG?) sprav	Yellow clouds
May 79	9	Nam Po	Plane (jet?) sprav	Yellow substance
May 79	9	Pha Mai	Plane (MIG?) spray, air burst	Yellow substance

C-6

UNCLASSIFIED

UNCLASSIFIED

ANNEX D

ANALYSIS AND REVIEW OF TRICHOTHECENE TOXINS

I. SAMPLE ANALYSES FOR TRICHOTHECENES

The Trichothecene Hypothesis

Since 1976, remarkably consistent reports detailing chemical attacks in Southeast Asia have been received by the Intelligence Community. Some of these reports were of particular interest in that they described the use of lethal agents producing symptoms that could not be correlated with those produced by traditionally recognized chemical warfare agents or combinations of them. Table D-1 is a compilation relating the signs and symptoms reported in Laos, Kampuchea, and Afghanistan with symptoms associated with certain chemical agents. The frequency with which a particular symptom was reported is expressed as a percentage of the total number of It is readily apparent that the symptoms most attacks. frequently described in Laos and Kampuchea correspond most closely with those produced by a group of mycotoxins, the trichothecenes. A review of the scientific literature revealed not only that these compounds had physical and chemical properties indicating potential as chemical agents, but also that they were the subjects of intensive investigation by Soviet scientists at institutes previously linked with chemical and biological warfare research. In the fall of 1980, the trichothecenes were added to the list of agents suspected to have been used in Southeast Asia and Afghanistan. Other candidates under consideration included phosgene oxime, arsines, cyanogen, nerve agents, riot control agents, and combinations of these agents.

Numerous samples from chemical attacks in Laos and Kampuchea were examined at the Chemical Systems Laboratory (CSL) for the presence of traditional chemical warfare agents and were reported to be negative. In March 1981 CSL reported the presence of an unusual compound $(C_{15}H_{24})$ in the vapor analyses from several clothing and tissue Samples taken from the victim of a chemical attack. The compound was very closely related in structure to the simple trichothecenes and this finding sparked the request for analysis of all future samples for the presence of trichothecene mycotoxins.

D-1

10

...)

Table D-1

Symptoms of Chemical Attacks Reported in Laos, Kampuchea, and Afghanistan

	Percentage							
	of Reports						Riot	
	Mentioning		Nerve		Phosgene		Incapitant	Control
Symptom	Symptom	Trichothecenes	Agents	Arsines	Oxime	Cyanogens	(BZ)	Agents
Laos					•	,		·
Multiple deaths	84.6	Х	X	X		х		
Vomiting	71.4	Х	X	X				Х
Diarrhea	53.1	Х	Х	Х		· • •		
Hemorrhage	52.0	Х			· X ^a	*-	** **	
Breathing difficulty	47.95	Х	Х	Х	Х	Х	Х	Х
Itching and skin								
irritation	43.9	Х		Х	X	~ ~		Х
Nausea	42.8	X	Х	Х		~-	Х	Х
Animal death	41.8	X	Х	Х		Х		
Blurred vision	39.7	Х	Х	· X	X	Х	Х	Х
Headache	36.7	Х	Х		X		X	Х
Fatigue	35.7	X	Х		~~		Х	
Nasal excretion	34.7	Х	Х	X	Х			Х
Rash or blisters	32.6	Х		Х	Х	· · ·		Х
Tearing	30.6	X	Х	Х	X	Х		Х
Coughing	28.6	Х	Х	Х	X	Х		Х
Effect on Vegetation	26.5	Х	- -	?	Х			
Dizziness and vertigo	25.5	Х	Х			Х	Х	Х
Facial edema	20.4	X		Х	Х	÷ •		Х
Thirst and dry mouth	20.4	Х					Х	 ,
Skin color change	16.3	Х			Х		~~	
Tachycardia	12.3	X	Х	-	Х	Х	Х	Х
Temporary blindness	9.18	Х		Х	Х		Х	Х
Rapid loss of								
consciousness	9.18	XD	Х			Х	Х	
Salivation	6.12	Xc	Х					
Hearing loss .	5.1	Х						
Tremors or convulsions	4	Х	Х		Х	Х		
Sweating	3		Х					
Paralvsis	3	X	X			Х		
Loss of appetite	3	X	X	Х				
Frequent urination	2	X	X					 .

D-la

Table D-1 (continued)

	Percentage							Piot
	Mentioning		Nerve		Phoseene		Incanitant	Control
Symptom	Symptom	Trichothecenes	Agents	Arsines	Oxime	Cyanogens	(BZ)	Agents
Kampuchea								
Multiple deaths	72.4	X	х	X		x		
Hemorrhage	62.06	X			xď			
Dizziness and vertigo	51.7	Х	х			х	х	х
Vomiting	41.3	Х	x	х				x
Nausea	34.5	Х	Х	X			x	x
Skin irritation	27.6	X		X	х			x
Rapid loss of								
consciousness	24.1	Xp	х	·		х	x	
Fever	20.68	X					***	· •••
Headache	17.2	X	х		х		· X	x
Tearing	13.8	X	X	х	x	x	x	x
Breathing difficulty	13.8	Х	х	X	X	X	x	x
Fatigue	13.8	Х	х				x	
Paralysis	10.3	Х	x			Х		
Numbness	6.9	Х	Х			X	х	?
Blurred vision	6.9	Х	х	· X	х	X	X	x.
Dry throat and thirst	6.9	Х					X	
Edema	6.9	X		х	Х			
Salivation	3.4	x ^e	X					
Vegetation affected	3.4	Х		?	?			
Diarrhea	3.4	Х	Х	X				
Cough	3.4	Х		X	х	X	x	x
Nasal discharge	3.4	X	х	X	x			x
Rash or blister	3.4	Х		X	X		+-	x
Chills	3.4	X	?					**
Hearing loss	3.4	Х		~-			~ -	~ ~

D-1b

Table D-1 (continued)

F	ercentage							
a	f Reports							Riot
M	entioning		Nerve		Phosgene		Incapitant	Control
Symptom	Symptom	Trichothecenes	Agents	Arsines	Oxime	Cyanogens	(BZ)	Agents
Afghanistan			·					
Rapid loss of		1						
consciousness	47.9	XD	Х	-		X	Х	
Skin irritation and itchin	g 31.5	Х		Х	Х		-	Х
Multiple deaths	30.1	Х	Х	Х		Х		
Nausea	20.5	Х	Х	Х	~ ~		Х	Х
Vomiting	19.1	Х	Х	Х				Х
Tearing	17.8	Х	Х	Х	Х	Х		Х
Dizziness and vertigo	16.4	Х	X			Х	Х	Х
Blisters or rash	15	Х		Х	Х			Х
Difficulty breathing	13.7	Х	Х	Х	Х	Х	Х	Х
Paralysis	13.7	Х	Х	~~ .	 '	Х		
Headache	12.3	Х	X		Х		X	Х
Temporary blindness	8.2	X		Х	Х		Х	Х
Salivation	6.8	X ^C .	Х					
Loss of appetite	6.8	Х	Х	Х				
Effects on vegetation	5.5	Х						
Fatigue	5	Х	Х				Х	
Confusion	4.1		Х				Х	~~
Hemorrhage	4.1	Х			X ^a			
Change in skin color	2.8	Х			Х			
Diarrhea	2.8	Х	Х	Х				
Coughing	1.3	Х	X	Х	Х	Х	Х	Х

D-lc

a Bloody frothing.
b Only at very high doses.
c Depending on which trichothecenes.
d Blood flecked frothing.
e Depending on compound.

.

<u>The Kampuchean Leaf and Stem Sample--The First Analysis</u> <u>for Trichothecenes</u>

On 24 March 1981 a number of samples from the US Embassy in Bangkok was received. Two of the samples were reported to have been collected from the site of a chemical attack that occurred in the vicinity of TV 3391, an area just south of Phnum Mak Hoeun. A vegetation sample and a water sample were collected within 24 hours of the attack. Examination of bodies of victims of this attack by medical personnel revealed highly unusual degeneration of the mucosal lining of the gastrointestinal tract. The effects described paralleled those known to be produced by the trichothecenes. The samples were submitted to Chemical Systems Laboratory for analysis for the presence of chemical warfare agents. No evidence of known chemical warfare agents was found. An initial test for the trichothecenes by thin layer chromatography (TLC) was inconclusive because of severe problems with interfering substances and the lack of appropriate standards. The trichothecenes are difficult to detect even under ideal circumstances and the presence of interfering substances in the sample may make identification and quantitation by TLC inconclusive. A review of the limitations and potentials of analytic methods for trichothecenes led those authors to conclude that the computerized gas chromatography/mass spectroscopy method in the selected ion monitoring mode would enable precise identification and quantitation of these compounds in complex mixtures. An additional recent publication includes a summary of the currently available methods suitable for trichothecene analysis and an assessment of their utility and limitations.

A portion of the leaf and stem sample was furnished to the US Army for further analysis. This sample (see table D-2, group I/A), a positive control sample to which T-2 toxin was added (group I/B), and a negative control sample of similar vegetation (group I/C) were forwarded to Dr. Chester J. Mirocha, Department of Plant Pathology, University of Minnesota. Dr. Mirocha was given no information concerning the history or content of the samples, and was requested to analyze the three unknowns for the presence of trichothecene toxins using the best methods at his disposal. Briefly, the analysis involves a series of extractions followed by ferric gel separation, selected ion monitoring on a computerized gas chromatograph/mass spectrometer, and a full mass spectral scan for comparison with known stand-

D-2

Table D-2

Trichothecene Sample Analyses

Reference Number, CASE Number	/ <u>Code</u>	Date Received	Description	Current Status
Sample Group I/ M-22-81		29 Apr 81	Leaf and stem + negative control	Analysis complete.
	А		Sample from attack area	Code A sample: T-2 - 3.15 ppm Nivalenol - 109 ppm Deoxynivalenol - 59.1 ppm
	В		(spiked) pos. control	Code B sample: T-2 - 35.7 ppm Nivalenol - 21 7 ppm
	С		External neg. control	Code C sample: Negative results
Sample Group II/ M-23-81	D	26 Aug 81	Environmental samples Water	Analysis complete. Code D sample: Deoxynivalenol - 66 ppm Diacetoxyscirpenol - Trace
	E ^a		Yellow powder	Code E sample: T-2 - 150 ppm Diacetoxyscirpenol - approx. 25 ppm
	F		Speck (unknown substance)	Code F sample: Diacetoxyscirpenol - 10 ng

^a Additional 25 mg of this sample was provided to laboratory for analysis for nivalenol and deoxynivalenol (see Sample Group X/M-11-82)

UNCLASSIFIED

D-2a

Table D-2 (continued)

Trichothecene Sample Analyses

Reference Number/ CASE Number	Code	Date Received	Description	Current Status
Sample Group III/		20 Sep 81	Environmental samples	Analysis complete.
M-20-01	Α		Water sample	Negative results on all samples tested to date.
	В		Soil sample	
	С		Soil sample	
	D		Dried corn	
	E		Rice	
	F		Leaves and stem	
	G		Leaves and stem	
	H		Leaves and stem	
	I		Leaves and stem	
Sample Group IV/ M-1-82		5 Oct 81	Environmental samples (Negative controls)	Retained at USAMIIA pending results of higher priority analyses.
Sample Group V/ M-2-82		11 Oct 81	Blood samples	Analysis complete.
	A14			Negative results on all
	A15			samples tested to date.
	A16	,		-
	A17			
Sample Group VI/ M-3-82		22 Oct 81	Blood samples	Analysis complete. Analytical findings to date have tentatively identified HT-2, a deacetylated

HT-2, a deacetylated metabolite of T-2 toxin, in the blood of patients 3 and 4 (see Table D-3).

· · · · · ·

UNCLASSIFIED

D-2b

L.

×.

Table D-2 (continued)

Trichothecene Sample Analyses

	Reference Number CASE Number	/ <u>Code</u>	Date Received	Description	Current Status
		A1 A13 B1	·		Medical laboratory evalua- tion of blood samples con- ducted by US Army Medical Research Institute of Infec- tious Diseases (USAMRIID) No significant statistical differences between control samples from alleged victims of ''yellow rain, but a trend toward low blood cell counts in victims was noted. See Table D-3
D-2c	Sample Group VII M-7-82	/	17 Nov 81	Blood samples	Retained at USAMIIA pending completion of higher priority analyses. A metabolite of T2 toxin (HT ₂) was tentatively identi- fied in the blood of two victims of a CW attack.
	Sample Group VII M-8-82	I/	17 Nov 81	Environmental samples	Analysis in progress.
	Sample Group IX/ M-9-82		6 Nov 81	Environmental samples (Sock, pants, mask, water)	Analysis in progress.
	Sample Group X/ M-11-82	E-2	19 Nov 81	Environmental sample Yellow powder	Analysis complete. 143 ppm T ₂ 27 ppm DAS 0 Nivalenol 0 Deoxynivalenol

UNCLASSIFIED

3

The methods used are among the most sensitive and ards. specific for detection of these compounds; also false positives are rare. Toxins can be identified by their mass spectra and quantified with a high degree of accuracy. Group I/A-the vegetation sample allegedly exposed to a CW agent--was found to contain 109 parts per million (ppm) of nivalenol, 59.1 ppm of deoxynivalenol, and 3.15 ppm of T-2 toxin; each is a potent toxin of the trichothecene group. No trichothecenes were detected in the negative control sample (group I/C), and 35 ppm of T-2 toxin were detected in group I/B--the sample to which T-2 toxin had been added. It was Dr. Mirocha's assessment that a mixture of these particular toxins in the high levels detected could not have occurred as a result of natural contamination.

The possibility that the identified toxins were produced by natural fungal contamination is addressed in section III. In summary, the possibility was discounted on the basis of the climatic conditions required for production of T-2 toxin, the high levels of toxins detected, the unusual mixture of toxins found, and the results of surveys of Southeast Asia for the presence of these toxins. This conclusion was supported by the analysis of normal flora samples from Kampuchea described below.

Analyses of Control Samples From Kampuchea for the Presence of Trichothecenes

On 20 September 1981, nine control samples were received from US Army personnel in Bangkok, Thailand, for the purpose of conducting laboratory analyses for background levels of trichothecene toxins. The samples were collected from an area near TV 3391 that had not been subjected to any reported chemical attacks. The samples were collected by US personnel under instructions to reproduce the sampling conditions, handling, packaging, and transfer conditions of the original sample as closely as possible. The same species of plant was sampled, and three other vegetation samples were also collected. A water sample as well as two soil samples Samples of corn and rice from the area were were recovered. also taken. These grains provide an ideal substrate for growth of toxin-producing fungi and would therefore be a sensitive indicator of any natural occurrence. The nine samples were forwarded under code to Dr. Mirocha for trichothecene analysis. A portion of each sample was also submitted to CSL for background determinations of CN-, C1-, and F1- levels. No trichothecenes were detected in any of these samples (group III/A-I), indicating that nivalenol,

D-3

deoxynivalenol, T-2, and diacetoxyscirpenol are not prevalent in the geographical area from which the alleged CW exposed sample was collected. The appearance of these trichothecenes in high levels and unique combinations in a sample associated with a chemical attack producing symptoms typical of trichothecenes exposure indicates that these toxins may have been used as chemical weapons. This conclusion is further supposed by the confirmatory evidence provided by the analysis of additional alleged CW samples from Laos and Kampuchea which are described below.

Analysis of Additional CW Samples From Laos and Kampuchea for the Presence of Trichothecenes

Chemical Systems Laboratory provided three additional suspected chemical warfare samples for analysis for tricho-The first sample (group II/D) consisted of 10 ml thecenes. of water taken from the same chemical attack site in Kampuchea as the leaf and stem sample previously examined (group I/A). The second sample (group II/E) came from the site of a ''yellow rain'' attack occurring on 13 March 1981 in the village of Muong Cha (TF 9797) in the Phou Bia region of Laos. The agent was sprayed from a twin-engine propeller aircraft at approximately 1200 hours. The falling substance was described as ''like insect spray'' and sounded like drizzling rain. It was quite sticky at first, but soon dried to a powder. Symptoms described by victims included nausea, vomiting, and diarrhea. A sample of the agent scraped from the surface of a rock by a victim and carried into Thailand was turned over to US Embassy personnel. The third sample (group II/F) was taken from the site of a ''yellow rain'' attack that occurred at 1400 hours on 2 April 1981 at Ban Thong Hak (TF 9177). Twenty-four people reportedly died in this attack and there were 47 survivors. Symptoms included severe skin irritation and rash, nausea, vomiting, and bloody diarrhea. This sample was scraped from the surface of a rock with a bamboo knife by a survivor of the attack. Although the individual took precautions (that is, cloth mask) a severe skin rash and blisters developed.

These three samples were submitted to Dr. Mirocha for analysis. Group II/D (the water sample from Kampuchea) contained 66 ppm of deoxynivalenol and a trace amount of diacetoxyscirpenol (DAS). A trace quantity of group II/E was screened as strong positive for trichothecenes. Further analysis of that sample confirmed the presence of high levels of T-2 toxin (150 ppm) and diacetoxyscirpenol (25 ppm).

D-4

T

Interference from phtalate compounds (leached from the plastic packaging) made detection of nivalenol and deoxynivalenol difficult. In a second analysis, the extraction process was modified so that nivalenol and deoxynivalenol could be measured accurately. The analysis showed the presence of 143 ppm T₂ and 27 ppm DAS. No nivalenol or deoxynivalenol was detected. ²Interestingly, examination of the petroleum ether fraction from sample group II/E revealed the presence of a yellow pigment almost identical to that previously identified by Dr. Mirocha in cultures of <u>Fusarium roseum</u>, indicating that the yellow powder probably consisted of the crude extract of a <u>Fusarium</u> culture.

There was very little of group II/F contained in the vial received for testing. The quantity was too small to be accurately weighed and inspection of the vial revealed only a very small speck estimated to weigh much less than 0.1 mg. That speck contained 10 ng of diacteoxyscirpenol, a level equivalent to 100 ppm at the very least and probably much higher. The sample size was too small to allow adequate analysis for the other three trichothecenes of interest.

These results, in general, support the hypothesis that trichothecenes have been used as chemical warfare agents in Laos and Kampuchea. The presence of these high levels of trichothecene toxins in water and in yellow powder scraped from rocks argue against natural occurrence, since neither water nor rock is a suitable environment for growth of the fungi required to produce the toxins.

Differences between the analyses of the Kampuchean leaf and stem sample and the water sample collected from the same attack site raise additional questions. The failure to find T-2 toxin in the water sample is probably due to the relative insolubility of T-2 toxin in water. The presence of DAS in the water might be the result of biotransformation or breakdown of T-2, as they are so structurally similar, differing only in the substitution on carbon 8. While this hypothesis cannot be entirely ruled out it is unlikely on the basis of known biotransformation of T-2 in the laboratory. The initial vegetation sample was not screened for DAS, though the mass spectra from the initial analysis will be reexamined for trace amounts of DAS.

The absence of nivalenol in the water sample is more difficult to explain because nivalenol is water soluble. The effect of environmental conditions and microorganisms on the stability of these compounds may vary widely for each of the specific compounds and may explain the analytical results. Further scientific investigation of these factors is needed.

D-5

٢.

II. ANALYSIS OF BLOOD SAMPLES FROM CHEMICAL ATTACK VICTIMS

Blood samples drawn from victims of recent chemical attacks have been received for analysis for indications of trichothecene exposure. Little is known concerning the rate of metabolism of trichothecenes in humans; it is difficult, therefore, to estimate the probability of detection of trichothecenes or their metabolites in blood samples. T-2 is rapidly cleared from the blood in animals, and 25 percent of the total dose is excreted within 24 hours after exposure; therefore, it is unlikely that trichothecenes could be detected unless samples were obtained within 24 to 48 hours after an Other blood parameters are affected by the trichotheattack. cenes, however, and may prove to be useful markers. The trichothecenes induce a severe leukopenia (decrease in white cell count) which can persist for several weeks following exposure. In addition, the trichothecenes affect some liver and kidney function marker enzymes which can be monitored in the blood.

On 11 October 1981, four whole blood samples and four blood smears were received from the US Embassy, Bangkok. The blood was drawn from four Khmer Rouge soldiers on 7 October inside Kampuchea. Detailed medical histories as well as descriptions of the attack were recorded on each individual from whom a blood sample was taken. All four men were victims of a gas attack occurring in the fall of 1981 near Takong. Symptoms experienced included vomiting, blurred vision, bloody diarrhea, difficulty breathing, dry throat, loss of consciousness, frontal headache, tachycardia, and facial edema. Unfortunately, the samples could not be refrigerated until 48 hours after collection. It was therefore not possible to obtain data concerning white cell counts and blood chemistry. The four whole blood samples were submitted to Dr. Mirocha for analysis for trichothecene metabolites because of the possibility (admittedly remote) that some of the metabolites may bind to blood proteins and may still be detectable even three weeks after an attack. These analyses are reported as group V A14-A17 in table D-2.

On 22 October 1981 additional blood samples were received. These had been drawn from nine victims from the 19 September attack and from four control individuals of similar age and background who had not been exposed to a chemical attack. The samples had been properly refrigerated and were accompanied by very complete and detailed medical histories taken by trained medical personnel who examined the individuals. Included in the package were blood smears and heparinized and nonheparinized samples from each individual. The samples were

D-6

5

submitted to US Army Medical Research Institute of Infectious Diseases (USAMRIID) for blood assays. These results are reported in table D-3.

The above results show no statistically significant differences between exposed and control groups (students T-test). A trend toward depressed white cell counts in eight individuals exposed to chemical agent was observed. Such an observation would be compatible with the clinical picture of toxin exposure; however, it is also compatible with a number of other medical problems and a larger control sample would be required before such results could be adequately interpreted. Abnormal liver and kidney functions were not indicated by this data.

Portions of these blood samples were analyzed by Dr. Mirocha for presence of trichothecenes and/or trichothecene metabolites. The results of those analyses are consistent with trichothecene exposure in at least two of the gassing victims and tend to support the hypothesis that a trichothecenebased agent was used in this attack.

Using the selected ion-monitoring gas chromatography/mass spectroscopy analysis technique, Dr. Mirocha was able to identify tentatively a metabolite of T₂ toxin (that is, HT_2) in the blood of two alleged victims. The compound was identified on the basis of its selected ion masses and gas chromatographic retention times.

The tentative identification of HT in the blood of two victims, and the trend toward depressed² white cell counts in these same victims, cannot be taken as conclusive scientific proof of toxin exposure because the trace amount of the compound present precluded unequivocal identification and quantitation, and also because many other medical problems in addition to toxin exposure can cause a decrease in white cell counts. It is interesting to note that the individual who showed the greatest amount of the compound tentatively identified as HT₂ in his blood, was reported to have received the greatest exposure to the agent and also had the lowest WBC. He was exposed to contaminated water for more than 30 minutes and was the only victim who fell down in the water and actually swallowed some of it. However, the description by victims of symptoms correlating exactly with those associated with trichothecene poisoning, provide strong circumstantial evidence that trichothecenes were used as chemical agents in yet another chemical attack in Southeast Asia.

D-7

UNCLASSIFIED

à

1,

Table D-3

Peripheral Blood Hemograms of Kampuchean Victims of Chemical Attack

Patient No.	RBC ^a	Hgb ^b	Hct ^C	WBCd	Retic	e MCV ^f	MCHg	MCHC ^h
1 2	specin 4.46	nen clo 12.6	tted 37	4,700	1.0	84	28.5	34
3	4.90	10.2	40	5,700	0.4	81 70	20	30
	4.90	15.0	46	5 300	1 2	93	32	34
6	4.04	12.6	37	4,300	0.8	93	31	34
7	4.88	15.6	46	3,000	0.5	94	32	34
8	5.56	17.0	50	8,700	1.5	91	31	34
. 9	4.88	11.2	35	5,000	1.0	73	23	32
Control	.s :							
10	6.23	12.5	41	7,200	0.8	66	20 ·	30
11	4.47	11.9	38	8,000	0.9	85	26.5	31
12	4.88	12.9	41	5,100	2.0	85	26.5	32
13	5.16	15.6	46	6,500	1.0	90	30.5	34
Normal male	range:						45 00	
female	.5-6.0	14-18	40-54	7,400		80-94	27-32	33–38
3	.5-5.0	12-16	37-47	±2,000				
		BU	N ¹	Creatinine	2	SGPT ^j	Alkalin Phospha	e tase
Normal	Range M F	7-	20	0.4-1.7		6-37	24-69 23-71	
	1.	9.	0	3.5		48	132	
	2.	8.	5	0.8		36	47	
	з.	8.	0	1.4		12	75	

D-7a UNCLASSIFIED

UNCLASSIFIED

Table D-3 (continued)

Peripheral Blood Hemograms of Kampuchean Victims of Chemical Attack

	BUNI	Creatinine	SGPT ^J	Alkaline Phosphatase
4A.	11	1.3	6	94
4B.	10.5	1.2	6	68
5.	6.0	1.6	12	84
6.	7	1.2	18	115
7.	8.5	1.7	6	69
8.	10	1.5	36	79
9.	12.5	1.4	12	70
10.	10.5	1.8	12	86
11.	12	0.8	24	74
12.	12	1.4	6	76
13.	9.0	1.2	30	102

Red blood cells $x10^{-6}$ (#/cc) а b Hemoglobin (gm/100cc) С Hematocrit (%) đ White blood cells (#/cc) е Reticulocytes (#/cc) f Mean corpuscular volume (u³) Mean corpuscular hemoglobin (uug) g h Mean corpuscular hemoglobin concentration (%) i

- Blood urea nitrogen (mg%)
- ^j Serum glutamic pyruoic transaminase

D-7b

UNCLASSIFIED

Trichothecenes have been identified previously in environmental samples taken from several other chemical attacks in Laos and Kampuchea. Analysis of control vegetation, water, soil, corn, and rice samples from these areas, as well as reviews of published scientific literature, indicate that the particular toxins that have previously been identified are not known to occur naturally in the combinations found and at the levels detected in Southeast Asia. The latest analysis results contribute another piece of evidence to the growing body of data supporting the charge that trichothecenes have been used as chemical/biological agents in Southeast Asia.

D-8

UNCLASSIFIED

III. OVERVIEW OF NATURAL OCCURRENCE AND SIGNIFICANT PROPERTIES OF TRICHOTHECENES

Historical Trichothecene Mycotoxicoses

The trichothecenes are members of a large group of naturally occurring toxins known as mycotoxins. The word ''mycotoxin'' is derived from the Greek ''mykes'' meaning fungus and the Latin ''toxicum'' meaning poison. It refers to a metabolite produced by a mold that is toxic to man or animals. Mycotoxicoses have been described as the ''neglected diseases'' and, before 1960, little English-language literature concerning the diseases caused by mycotoxins was available. Interestingly, the first comprehensive studies of mycotoxin diseases were conducted in the Soviet Union in the late 1930s. Thus, Soviet scientists have been involved in research with some of these compounds for almost 30 years longer than their Western counterparts (see section V of this annex). The Soviet Union has had serious problems with mycotoxin contamination of food and has suffered several severe outbreaks of disease in humans.

The group of mycotoxins that has figured most prominently in Soviet scientific literature since the 1940s are the trichothecenes. They are a group of chemically related, biologically active fungal metabolites produced primarily by various species of <u>Fusarium</u>. Table D-4 lists some of the toxins in this group and producing fungi. The fungi are well-known plant pathogens that frequently invade numerous agricultural products.

Trichothecene toxins, perhaps more than any other mycotoxins, have been associated with acute disease in humans. Most of the human intoxications occurred in the Soviet Union (table D-5). The earliest recognized outbreak occurred in 1891 in the Ussuri district of eastern Siberia. Humans who consumed contaminated grain exhibited headache, chills, nausea, vomiting, vertigo, and visual disturbances. Dogs, horses, pigs, and domestic fowls were reported to be affected.

The most extensive mycotoxicosis outbreak reported to have caused multiple fatalities in man also occurred in the Soviet Union. In 1944 30 percent of the population of Orenburg near Siberia was affected by alimentary toxic aleukia or ATA, a disease later shown to be due to ingestion of trichothecene toxins. Over 10 percent of the entire population of the Orenburg district died of the disease. Numerous other

> D-9 UNCLASSIFIED

1

TABLE D-4

Trichothecene-Producing Fungi

Туре	(A) T-2 Type	(B) Nivalenol-Type	(C) Macrocylic
	T ₂ Toxin	Nivalenol	Roridins
Trichothecenes	HT ₂ Toxin	Monoavetyl- Nivalenol	Veirucarins
	Diacetoxy- Scirpenol	Diacetgl- Nivalenol	Satratoxins
	Neosolaniol	Deoxynivalenol	Vertisporin
	F. tricinctum	F. nivale	Myrothecium verrucaria
Fungus	F. roseum	F. opisphaeria	
			M. roridum
	F. equiseti	F. roseum	
	F. sporotrichioide	S	Stachybotrys atra
	F. lateritium		Verticimonosporium diffractum
	F. poae		
	F. solani		
	F. rigidiusculum		
	F. semitectum		

TABLE D-5

Historical Trichothecene Mycotoxicosis

Toxicosis

''Taumelgetreide'' Toxicosis

Alimentary toxic aleukia

Stachibotryotoxicosis

Bean-hull toxicosis

Dendrodochiotoxicosis

Moldy corn toxicosis

Red mold toxicosis

Districts and Affected Species

USSR Man, farm animals

> USSR Man, horse, pig

USSR, Europe Horse

> Japan Horse

USSR, Europe Horse

United States Pig, cow

Japan, USSR Man, horse, pig, cow

Symptoms

Headache, nausea, vomiting, vertigo, chills, visual disturbances

Vomiting, diarrhea, multiple hemorrhage, skin inflamation, leukopenia, angina

Shock, somatitis, hemorrhage, dermal necrosis, nervous disorders

Convulsion, cyclic movement

Skin inflamation, hemorrhage

Emesis, hemorrhage

Vomiting, diarrhea congestion and hemorrhage of lung and intestine

UNCLASSIFIED

outbreaks of ATA occurred in the Soviet Union, primarily between the years 1942-1947. The contamination was traced to overwintered millet, wheat, and barley infected with Fusarium. Symptoms of the disease included vomiting, skin inflammation, multiple hemorrhaging (especially of the lung and gastrointestinal tissue), diarrhea, leukopenia, and suppression of bone marrow activity.

In 1939, Nikita Khrushchev was dispatched to the Ukraine region of Russia by Premier Joseph Stalin to organize and improve agricultural operations and to identify the disease that was causing the deaths of many horses and cattle. The problem was traced to hay and straw contaminated with <u>Stachybotrys atra</u>. The disease, later referred to as stachybotryotoxicosis, occurred after ingestion or contact with the contaminated grain. Symptoms included ulcerative dermatitis, perioral dermatitis, blood dyscrasias, hemorrhagic syndromes, abortion, and death. The greatest economic impact was due to loss of horses, but cattle, sheep, poultry, and humans were also affected.

Other disease outbreaks in which similar symptoms were exhibited occurred in 1958 and 1959 among horses and cattle in the Soviet Union and Eastern Europe. Thousands of animals were lost in these outbreaks. Other intoxications were reported subsequently in Japan, Europe, the Soviet Union, and the United States, affecting various domestic animals and--in the case of red mold toxicosis--affecting man. All of these diseases have now been shown to be due to ingestion of trichothecenes rather than to an infectious agent. In earlier disease outbreaks, the levels of toxin present in the contaminated grain was not measured; however, the levels of nivalenol and/or deoxynivalenol measured in toxic grains implicated in more recent outbreaks (that is, ''moldy corn toxicosis'' and ''red mold toxicosis'') were typically between 2 and 8 ppm.

Natural Occurrence of Trichothecene Mycotoxins

Publications concerning the occurrence of trichothecenes have been relatively scarce because of the lack of convenient detection methods and the complexity of the trichothecene family of compounds. Only recently have scientists developed methods capable of distinguishing between close structural derivatives and accurately quantitating the levels of toxin present (see table D-6 for comparison of analytical methods). Extreme care must be taken when reviewing the scientific

> D-12 UNCLASSIFIED

TABLE D-6

Physiochemical Methods for Detection of Trichothecenes in Feedstuffs

Method	Irichothescenes Detected	Detection Limits	Required Standards	Use and Limitation
Thin-layer chromatography 1-dimension	All	0.1 micogram/spot (H ₂ SO ₄)	Reference Standard	Qualitative Interference Not confirmatory
Thin-layer chromatography 2-dimension	All	0.1-1.0 microgram/ spot (H ₂ SO ₄)	Reference Standard	Qualitative Less interference Confirmatory
└ Gas-liquid └ chromatography	Nonhydroxy- lated or TMS derivatives	0.03-0.05 microgram/ microliter injection	Reference Standard	Quantitative Monoglyceride interference Equivocable identification
Gas chromato- graphy/mass spectrometry- normal scanning mode	TMS derivatives	0.02-0.05 microgram/ microliter injection	Reference Standard or Spectrogram	Semiquantitative Less interference Unequivocable identification
Gas chromato- graphy/mass spectrometry- selection ion monitoring	TMS derivatives	0.007-0.02 microgram/ microliter injection	Reference Standard or Spectrogram	Quantitative Best for complex mixtures Unequivocable identification
Nuclear-magnetic resonance	c- All		Reference Standard or Spectrogram	Confirmatory Purified toxin structure elucidation

TABLE D-6 (continued)

Method	Trichothescenes Detected	Detection Limits	Required Standards	Use and Limitation	~~ ~~ .
Radioimmunoassa (developmental stage)	y T-2 toxin	1-20 nanogram	Rabbit anti- T-2 toxin anti-body	Sensitive Low inter- ference	
			³ H-T-2 toxin	Relative structural specificity	

UNCLASSIFIED

Approved for Release: 2015/01/05 C05184036

. . .

literature on natural occurrence of these compounds, because erroneous conclusions can be drawn on the basis of results obtained with inadequate analytical techniques. Misidentification of compounds and gross overestimation of concentrations have occurred using techniques such as thin layer chromatography as the basis of analysis. Table D-7 lists the reports of natural occurrence of T-2 toxin, diacetoxyscirpenol, and nivalenol that were obtained from a literature search of over 3,000 citations concerned with trichothecene toxins. Levels that are questionable on the basis of techniques used are indicates. It is immediately apparent that the levels of toxins found in the various samples from Laos and Kampuchea are highly unusual, even if one accepts the questionable reports in table D-7 as valid. The levels of these toxins (150 ppm T-2 toxin, 109 ppm of nivalenol, more than 100 ppm of diacetoxyscirpenol, and 66 ppm of deoxynivalenol) are markedly higher than those reported to occur in nature. It should also be noted that the incidences recorded in table D-7 concern levels of toxin produced when **Fusarium** is growing on its ideal substrate, while the Laos and Kampuchea samples were taken from surfaces that would be extremely unlikely to support Fusaria growth and toxin production, that is, the surface of rocks and water. Higher levels of toxin production can, of course, be induced when the mold species is grown in pure culture under ideal conditions in the laboratory; for instance, the Soviets have succeeded in producing 4 grams of T-2 per kilogram of sub-In a natural environment, however, the Fusaria strate. species cannot compete well with other molds such as species of Aspergillus and Penicillium, and, as in table D-7, levels of toxin produced are orders of magnitude lower.

The conclusion that the levels of toxins found in the Southeast Asia samples could have occurred only by means of an unnatural mechanism is also strengthened by surveys of the area conducted by various researchers. Surveys of the toxigenic fungi and mycotoxins of Southeast Asia conducted by the Mahidol University in Bangkok and the Massachusetts Institute of Technology, have not revealed the presence of T-2, nivalenol, deoxynivalenol, or diacetoxyscirpenol, although other mycotoxins such as aflatoxin, were identified. These results were confirmed by our analysis using our own methodology of normal flora samples of vegetation, soil, water, corn, and rice from Kampuchea that revealed the presence of no trichothecenes.

D-15

UNCLASSIFIED

Approved for Release: 2015/01/05 C05184036_

ъ

Table D-71. Spontaneous Occurrence of Trichothecene Mycotoxins

Toxin	Country	Source	Concentration (parts per million)	Reference ^a
T-2 Toxi	in			
	USA	Mixed feed	0.08 ^b	15
	UK	Brewer's grains	ND ^C ,	19
	India	Sweet corn	4 ^{b,d}	5
	Canada	Corn	ND,	4
	India	Sorghum		22
	Canada	Barley	25 [°] ,	20
	India	Safflower seed	3-5 ⁴	6
	US	Corn stalks	0.11^{D}	16
	US	Feed supplement	ND	7
	US	Corn	2	8
	US	Mixed feed	0.3 b	14
	France	Corn	0.02	10
	US	Corn	ND	2
Diacetox	xyscirpenol			
	US	Mixed feed	0.5	15
	US	Mixed feed	0.38	15
	India	Safflower seed	3-5	6
	India	Sweet corn	14 ⁰	5
	Germany	Corn	31.5 ^u	23
	US	Corn	0.88	21
Deoxyniv	valenol			
	US	Corn stalks	1.5 ^b	16
	US	Corn	1.8 ^D	15
	US	Corn	1.0^{D}_{L}	15
	US	Corn	0.1^{D}_{h}	15
	US	Mixed feed	0.04	15
	US	Mixed feed	1.0 ^b	15
	US	Mixed feed	1.00	15
	US	Corn	7.4 d	9
	US	Corn	0.1-25 [°] d	21
	US	Corn	trace-25"	2, 21

D-16

UNCLASSIFIED

Table D-7 (continued)

Toxin	Country	Source	Concentration (parts per million)	Reference ^a
Deoxyniv	valenol (Continued)			
	US	Corn	1.1-10.7	26
	US	Corn	41 b	25
	US	Corn	1,00	17
	US	Oats	5	17
	Japan	Barley	ND b	18
	US	Corn	1.0 ^b	13
	US	Corn	$0.06_{\rm b}^{\rm S}$	13
	US	Mixed feed	0.075	13
	France	Corn	0.6	10
	South Africa	Corn	2.5	11
	Zambia	Corn	. 7.4	11
	US	Corn	ND	2
	Japan	Barley	7.3	18
	Austria	Corn	1.3	24
	Austria	Corn	7.9	24
	Canada	Corn	7.9	24
Nivalen	<u>ol</u>		•	
	Japan	Barley	ND b	18
	France	Corn	4.3	10
Partial	ly characterized tr	ichothecenes		
	US	Corn	ND	25
	India	Safflower seed	ND ^d	6
<u>Skin ir</u>	ritant factors-not	analyzed chemically	· · · · · · · · · · · · · · · · · · ·	
	US	Corn	93 positive $^{\rm b}$ of 173	3
	US	Corn	Multiple positive sample	≥s 21
	Yugoslavia	Corn	16 positive of 191	1

D-17

UNCLASSIFIED

_Approved for Release: 2015/01/05 C05184036

,

UNCLASSIFIED

а References:

ъ

			h h
1.	Balzer et al. (1977)	14.	Mirocha (1979 ^D) b
2.	Ciegler (1978)	15.	Mirocha et al. (1976°)
3.	Eppley et al. (1974)	16.	Mirocha et al. $(1979_{\rm h}^{\rm a})$
4.	Funnel (1979)	17.	Mirocha et $\overline{a1}$. (1979 ^b)
5.	Ghosal et al. (1978)	18.	Morooka et al. (1972)
6.	Ghosal $\overline{\text{et al}}$. (1977)	19.	Petrie et al. (1977)
7.	Hibbs et al. (1974)	20.	Puls and Greenway (1976)
8.	Hsu et al. (1972)	21.	Romer, T., Ralston Purina, St. Louis, MO
			(personal communication)
9.	Isshi et al. (1975)	22.	Rukmini and Bhat (1978)
10.	Jemmail et al. (1978)	23.	Siegfried (1979)
11.	Marasas et al. (1977)	24.	Vesonder and Ciegler (1979)
12.	Miller (1976)	25.	Vesonder et al. (1976)
13.	Mirocha (1979 ^ª)	26.	Vesonder \overline{et} \overline{al} . (1978)

b

С

Zearalenone (F-2 Toxin) also detected in the sample. ND = toxin concentration was not determined. Levels that are questionable on the basis of techniques used. d

D-18 UNCLASSIFIED

Skeptics have formulated theoretical explanations for the analytical results to support a hypothesis of natural occurrence of these toxins. It was postulated that the trichothecenes found were absorbed through the roots of a plant, translocated to the leaves, and exuded and washed onto the surface of a rock and into water where they were found. 1981 publication by Jarvis et al. reported a Brazilian shrub that appeared to absorb, translocate, and chemically alter a macrocyclic trichothecene produced by a soil fungi. While this citation is used to support a hypothetical mode for natural deposition in Southeast Asia it should be noted that the plant reported by Jarvis et al. did not exude the toxin, that the toxin was extremely phytotoxic to all other plants assessed, and that the plant was not capable of denovo trichothecene synthesis. No other trichothecenes have been found to be absorbed and translocated in any other plant in this manner. Control samples of soil and vegetation from Southeast Asia do not support endemic presence of these toxins. The appearance of these particular trichothecene toxins in these high levels in environments generally inhospitable to their formation cannot reasonably be attributed to a natural contamination.

Chemical and Physical Properties of the Trichothecenes

When considering the suitability of trichothecenes as agents, factors such as stability, solubility, and ease of production must be considered. The general structure for the trichothecene group is shown in figure D-1. There are over 40 currently known, naturally occurring, 12 to 13 epoxytrichothecenes. The R groups may be hydroxyls, acylated hydroxyl groups, or esters. The R group for the toxins detected in Sample Group I/A are shown below the general structure. All of the compounds have in common an olefinic double bond at carbon atoms 9 and 10 and an epoxy group at carbon atoms 12 and 13. These compounds are stable, especially in the solid form. They may be stored for years at room temperature with no loss of activity. They are heat stable with no loss of activity noted after heating for 1 hour at 100 degrees centigrade. The solubility depends on the R groups; highly hydroxylated derivatives are more water soluble. The compounds are also quite stable in solution. Detoxification can be accomplished by treatment with strong mineral acid, which will open the 12 to 13 epoxide bond and abolish all biological activity. Most of the toxins are well absorbed through mucous membranes and some through skin; this property is also a function of the R group.

D-19

UNCLASSIFIED

Figure D-1

General Structure of Trichothecenes



Some of these compounds have been synthesized chemically; however, biosynthesis employing Fusarium species is the most effective way to produce large quantities. In a preliminary search of recent Soviet open source literature, 50 articles dealing with the trichothecenes were reviewed. Of these, 22 dealt with defining optimum conditions for biosynthesis of the compounds. N. A. Kostyunina has reported production of T-2 toxin at levels of 4 grams per kilograms of substrate (normally wheat grain, or rice). Numerous industrial microbiology plants have been identified in the Soviet Union. Some of these are involved in production of single-cell protein for fodder additives, others produce antibiotics, and . the function of still others is unknown. The only difference between an antibiotic and mycotoxin is their target specificity. Both are produced by fungi, but the mycotoxins are relatively more toxic to man than to microorganisms. Mycotoxins can be produced in good yield employing the same techniques that are used to produce some antibiotics. Thus, it may be concluded that the Soviets could produce trichothecenes in large amounts. They produce an antibiotic that is a trichothecene derivative, which would provide an ideal cover for agent production facilities.

Medical Effects of the Trichothecenes in Humans

The most prominent symptoms associated with trichothecene poisoning are listed in table D-5. Striking among these is the rapid onset of vomiting with severe itching and tingling of the skin. Hemorrhage of the mucous membranes and bloody diarrhea follow. That table also presents symptoms reported to have been caused by the trichothecenes in gas attack victims in Laos, Kampuchea, and Afghanistan. The correlation is striking.

The LD_{50} 's (dose required to produce death in 50 percent of a test population) of the trichothecenes in laboratory animals range from 0.1 mg/kg to greater than 1,000 mg/kg depending on the particular toxin, species, and route of exposure. The LD_{50} of T-2 toxin in cat is 0.5 mg/kg. However, the ED_{50} (dose required to produce a desired physiological effect in 50 percent of a test population) is much lower. The ED_{50} to produce a vomiting reaction is 0.1 mg/kg, and for skin irritation it is in the tenths of microgram range.

> D-21 UNCLASSIFIED

UNCLASSIFIED

Most of the data concerning the toxicological effects of the trichothecenes are derived from animal data in which pure compounds were administered by oral, subcutaneous, intraperitoneal or intravenous routes. Unfortunately, there are no reports concerning the effects of inhalation of mixtures of the compounds. Therefore, it is difficult to speculate concerning the effects that would be expected in humans who were exposed to an aerosol of mixtures of these very potent The most useful data concerning exposure in humans toxins. was obtained in a Phase I clinical evaluation of anguidine (diacetoxyscirpenol) as an anticancer drug. Diacetoxyscirpenol was administered by intravenous infusion. Doses of 3 mg/m²/day caused immediate onset of nausea, vomiting, diarrhea, somnolence (and/or mental confusion), fever, chills, a generalized erythema with a burning sensation, hypotension, dyspnea, stomatitis, hives, and ataxia. Because of the side effects the treatment was discontinued. The properties which make the use of diacetoxyscirpenol potentially useful as an anticancer drug are the same as those responsible (in part) for its extreme toxicity. It and the other trichothecenes cause extensive damage to rapidly dividing cells such as tumor cells. Unfortunately, the cells of the lining of the gastrointestinal tract and bone marrow are also rapidly dividing and the effects of the trichothecenes on these cells result in severe rapid degeneration of these tissues. The compounds also have direct effects on the clotting factors in the blood (that is, a primary effect on Factor VII activity and a secondary effect on prothrombin) which result in excessive hemorrhage following trauma.

The other useful body of clinical data concerning the effects of trichothecenes in humans is drawn from descriptions of the course of the disease in the natural outbreaks that occurred in the Soviet Union. The clinical picture may be divided into four stages. The effects produced are very similar to radiation poisoning and there is a latent phase in which the overt symptoms disappear similar to that seen in radiation poisoning.

The first stage occurs within minutes to hours after ingestion of toxic grains. The symptomatology described was produced by oral exposure to low doses. In exposure by inhalation, the symptoms may be more pronounced or the time course accelerated. The characteristics of the first stage include primary changes, with local symptoms, in the

D-22

UNCLASSIFIED

buccal cavity and gastrointestinal tract. Shortly after ingestion of toxic grain, the patient experiences a burning sensation in the mouth, tongue, throat, palate, esophagus, and stomach as a result of the toxin's effect on the mucous membranes. The tongue may feel swollen and stiff and the mucosa of the oral cavity may be hyperemic. Inflammation of the gastric and intestinal mucosa occurs, along with vomiting, diarrhea, and abdominal pain. In most cases excessive salivation, headache, dizziness, weakness, fatigue, and tachycardia accompany the initial stage. There may be fever and sweating, but the body temperature normally does not rise. The leukocyte count may begin to decrease in this stage and there may be an increased erythrocyte sedimentation rate. This first stage may last from three to nine days.

The second stage is often called the latent stage or incubation period because the patient feels well and is capable of normal activity. It is also called the leukopenic stage because its main features are disturbances in the bone marrow and the hematopoietic system, characterized by a progressive leukopenia, a granulopenia, and a relative lymphocytosis. In addition, anemia and a decrease in erythrocytes, in the platelet count, and hemoglobin occurs. Disturbances in the CNS and autonomic nervous systems may occur. Weakness, vertigo, fatigue, headache, palpitations, and mild asthmatic conditions may occur. Visable hemorrhagic spots (petechiae) begin to appear on the skin and this marks the transition to the third phase. The second stage may last three to four weeks. The transition to the third stage is sudden and symptoms progress rapidly.

In the third stage petechial hemorrhages occur on the skin of the trunk, arms, thighs, and face and head. They can vary from a millimeter to a few centimeters in size. Capillaries are very fragile and any slight trauma results in hemorrhage. Hemorrhages of the mucous membranes of the mouth, tongue, soft palate, and tonsils occur. Nasal, gastric, and intestinal hemorrhages can be very severe. Areas of necrosis begin to appear on the lips, fingers, nose, jaws, eyes, and in the mouth. Lymph nodes are frequently enlarged and the adjoining connective tissue can become so edematous that the patient has difficulty opening his mouth. Blood abnormalities previously described are intensified. Death may occur from hemorrhage, strangulation (due to swelling) or secondary infection.

D-23

UNCLASSIFIED

The fourth stage is convalescence. Three to four weeks of treatment are required for disappearance of necrotic lesions and hemorrhagic effects. Two months or more may elapse before the blood forming capability of the bone marrow returns to normal.

D-24

UNCLASSIFIED

IV. Bibliography of Literature Non-Soviet

Angsubhakorn, S., Sahaphong, S., Phiernpichit, L., Romruen, K., Thamavit, W., and Bhamarapravati, N., ''Toxigenic Fungi in Food and Foodstuffs of Thailand,'' J. Med. Ass. Thailand, Vol. 60, No. 4, (1977), pp. 162-168.

Balzer, I., Bodanic, C., and Muzic, S., ''Natural Contamination of Corn (Zea mays) with Mycotoxins in Yugoslavia,'' Annals Nutrition Alimentals, Vol. 31, 1977, pp. 425-430.

Bamburg, J. R. and Strong, F. M. ''Mycotoxims of the trichothecene family produced by <u>Fusarium tricinctum</u> and <u>trichoderma</u> <u>lignorum</u>, ''Phytochemistry, Vol. 8, 1969, pp. 2405-2410.

Ciegler, A., ''Trichothecenes: Occurrence and Toxicoses,'' Journal of Food Protecion, Vol. 41, 1978, pp. 399-403.

Dekker, ''Mycotoxic Fungi, Mycotoxins, Mycotoxicoses,'' An Encylopedic Handbook, 1977.

Eppley, R. M., Stoloff, L., Trucksess, M. W., and Chung, C. W., ''Survey of Corn for Fusarium Toxins,'' Journal of the Association of Official Analytical Chemists, Vol. 57, 1974, pp. 632-635.

Funnel, H. S., ''Mycotoxins in Animal Feedstuffs in Ontario 1972 to 1977, ''Canadian Journal of Comparative Medicine, Vol. 43, 1979, pp. 243-246.

Ghosal, S., Chakrabarti, D. K., and Choudhary, K. C. B., ''The Occurrence of 12, 13 Epoxytrichothecenes in Seeds of Safflower Infected with Fusarium oxysporium f. sp. carthami,'' Experientia, Vol. 33, 1977, pp. 574-575.

Ghosal, S., Biswas, K., Srivastava, R. S., Chakrabarti, D. K., and Choudhary, K. C. B., ''Toxic Substances Produced by Fusarium V: Occurrence of Zearalenone, Discetoxyscirpenol and T-2 Toxin in Moldy Corn Infected with Fusarium moniliforme Sheld,'' Journal of Pharmaceutical Science, Vol. 67, 1978, pp. 1,768-1,769.

Hibbs, C. M., Osweiler, G. D., Buck, W. B., and Macfee, G. P., ''Bovine Hemorrhagic Syndrome Related to T-2 Mycotoxin,'' Predeedings of the American Association of Veterinary Laboratory Diagnosticians, Vol. 17, 1974, pp. 305-310.

D-25

UNCLASSIFIED

Approved for Release: 2015/01/05 C05184036_

Hsu, I. C., Smalley, E. B., Strong, F. M., and Ribelin, W. E., ''Identification of T-2 Toxin in Moldy Corn Associated with a Lethal Toxicosis in Dairy Cattle, '' Applied Microbiology, Vol. 24, 1972, pp. 684-690.

Ishii, K., Ando, Y., and Ueno, Y., ''Toxicological Approaches to the Metabolites of Fusaria. Isolation of the Vomiting Factor from Moldy Corn Infected with Fusarium Species, '' Chemical Pharmacology Bulletin, Vol. 23, 1975, pp. 2,162-2,164.

Jarvis, B. B., Midiwo, T. O., and Tuthill, D., ''Interaction Between the Antibiotic Trichothecenes and the Higher Plant Baccharis megapotamica, '' Science, Vol. 214, 1981, pp. 460-461.

Jemmali, M., Ueno, Y., Ishii, K., Frayssinet, C., and Etienne, M., ''Natural Occurrence of Trichothecenes (nivalenol, deoxynivalenol, T-2) and Zearalenone in Corn, '' Experientia, Vol. 34, 1978, pp. 1,333-1,334.

Joffe, A. Z. ''Alimentary Toxic Aleukia,'' in <u>Microbial</u> <u>Toxins</u>, Vol. 7, 1971, pp. 139-189. Edited by S. Kadis, A. Ciegler, S. J. Ajl, Academic Press, N. Y.

Marasas, W. F. O., Kreik, W. P. J., Van Rensburg, S. T., Steyn, M., and Van Schalkwyk, G. C., ''Occurrence of Zearalenone and Deoxynivalenol, Mycotoxins Produced by Fusarium graminearum Schwabe in South Africa, '' South African Journal of Science, Vol. 73, 1977, pp. 346-349.

Miller, J. K., ''Suspected Mycotoxic Diseases of Pigs in Scotland. Second Meeting on Mycotoxins in Animal Disease, '' Eds. Patterson, D. S. P., Pepin, G. A., and Shreeve, B. J., 1976, pp. 14-16. Pinner: Middlesex.

Mirocha, C. J., ''Fusarium Species and their Effects on Farm Animals,'' Proceedings of the 15th Annual Nutrition Conference for Feed Manufacturers, 1979a, pp. 49-58, Guelph.

Mirocha, C. J., ''Trichothecenes Produced by Fusarium. Conference on Mycotoxins in Animal Feeds and Grains Related to Animal Health, '' Ed. Himoda, W., 1979b, pp. 289-260. Report FDA/BVM-79/139. National Technical Information Service: Springfield.

Miocha, C. J., Pathre, S. V., Schauerhamer, B., and Christensen, C. M., ''Natural Occurrence of Fusarium Toxins in Feedstuff, '' Applied and Environmental Microbiology, Vol. 32, 1976b, pp. 553-556.

D-26

UNCLASSIFIED



£

UNCLASSIFIED

Morooka, N., Uratsuji, N., Yoshizawa, T., and Yamamoto, H., ''Studies on the Toxic Substances in Barley Infected with Fusarium,'' Japanese Journal of Food Hygiene, Vol. 13, 1972, pp. 368-375.

Murphy, W. K., Burgess, M. A., Valdivieso, M., Livingston, R. B., Bodey, G. P., and Freireich, E., ''Phase I Clinical Evaluation of Anguidine,'' Cancer Treatment Reports, Vol. 62, 1978, pp. 1,497-1,502.

Petrie, L., Robb, J., and Stewart, A. F., ''The Identification of T Toxin and its Association with a Hemorrhagic Syndrome in Cattle,'' Veterinary Record, Vol. 101, 1977, p. 326.

Puls, R., Greenway, J. A., ''Fusariotoxicosis from Barley in British Columbia II. Analysis and Toxicity in Suspected Barley,'' Canadian Journal of Comparative Medicine, Vol. 40 1976, pp. 16-19.

Rukmini, C., Bhat, R. V., ''Occurrence of T-2 Toxin in Fusarium-infested Sorgham from India,'' Journal of Agriculture and Food Chemistry, Vol. 26, 1978, pp. 647-649.

Shank, R. C., Wogan, G. W., and Gibson, J. B., ''Toxigenic Molds in Foods and Foodstuffs in Tropical South-east Asia,'' Fd. Cosmet. Toxicol., Vol.10, 1972, pp. 51-60.

Siegfried, R., ''Fusarium-toxine,'' Naturwissenschaften, Vol. 64, 1977, p. 274.

Vesonder, R. F., Ciegler, A., ''Natural Occurrence of Vomitoxin in Austraian and Canadian Corn,'' European Journal of Applied Microbiology and Biotechnology, Vol. 8, 1979, pp. 237-240.

Vesonder, R. F., Ciegler, A., Jensen, A. H., Rohwedder, W. K., and Wiesleder, D., ''Co-identity of the Refusal and Emetic Principle from Fusarium-infected Corn,'' Applied and Environmental Microbiology, Vol. 31, 1976, pp. 280-285.

Vesonder, R. F., Ciegler, A., Rodgers, R. F., Burbridge, K. A., Bothast, R. J., and Jensen, A. H., 'Survey of 1977 Crop year Preharvest Corn for Vomitoxin,' Applied and Environmental Microbiology, Vol. 36, 1978, pp. 885-888.

D-27

UNCLASSIFIED
UNCLASSIFIED

Soviet Scientists Involved in Mycotoxin Research

A. Kh. Sarkisov

V. I. Bilay -(also spelled Bilai) V. A. Tutel'yan -

M. A. Akhmeteli

All Union Scientific Research Institute of Experimental Veterinary Science, Moscow Ukrainian SSR Institute of Microbiology and Virology, Kiev USSR Academy of Medical Sciences Nutrition Institute, Moscow USSR Academy of Medical Sceinces Institute of Epidemiology and Microbiology

L. Ye. Olifson M. F. Nesterin K. Z. Salomatina Ye. P. Kozhevnikova N. D. Osadchaya L. F. Mikhaylova Sh. M. Kenina V. L. Kartashova L. R. Filonova T. Ye. Tolcheyeva Kn. A. Dzhilavyan I. S. Yelistratov N. S. Tishkova V. I. Kaplun Ye. Pl Kozhevalkova S. M. Gubkin L. I. Il'ina P. A. Il'in

- A. M. Kogan
- D. T. Martynenko
- N. A. Kostyunina
- V. V. Yerinakov
- I. A. Kurmanov
- V. V. Semenov
- Z. K. Bystryakova
- Z. Z. Orlova
- L. S. L'vova
- L. I. Lozbina
- T. A. Shevtsova
- I. Yu Makedon
- N. S. Proskuryakova
- A. V. Borovkov
- M. N. Nazypov
- L. I. Lozbin
- M. S. Marova

D-28

V. SELECTED BIBLIOGRAPHY OF SOVIET LITERATURE

а

Maksimova, R. A., Palmova, N. P., Khuratova, B. G., ''The Effect of Polyploidogenous Factors on the Mycelium of Trichothecin and Fibrinolytic Enzymes,'' Mikrobiologiya, 1979, Vol. 48, No. 2, pp. 324-328.

Zhakhanov, A., ''Growth and Development of Weakly and Strongly Toxic Strains of Fusarium Sporotrichioides With Different Sources of Nitrogen Nutrition,'' Vestnik Sel'skokhozyaistvennoi Nauki Kazakhstana, 1977, Vol. 20, No. 9, pp. 35-36.

Kirpichenko, L., ''Effect of Different Sources of Nitrogen on the Growth and Pathogenicity of Fusarium Oxysporum,'' Referativnyi Zhurnal, 1976, 3.14.434.

Berestetskii, O. A., Nadkernichnii, S. P., Patyka, V. F., ''Isolation and Characteristics of a Phytotoxic Substance Produced by Fusarium Lateritium Nees,'' Mikologiya i Fitopathologiya, Vol. 9, No. 4, 1975, pp. 325-327.

Paletskaya, L. N., Kiseleva, N. T., Zhuravleva, V. P., Gorina, E. I., Saryeva, A. N., ''Effects of Nitrogenous Fertilizers on Fusarium Oxysporum f. Vasinfectum and Its Toxicity,'' Mikologiya i Fitopatologiya, Vol. 7, No. 6, 1973, pp. 515-520.

Kvashnina, E. S., ''Physilogical and Ecological Characteristics of Fusarium Species of the Section Sporotorichiella,'' Mikologiya i Fitopatologiya, Vol. 10, No. 4, 1976, pp. 275-282.

Kalinina, R. T., ''The Determination of Pathogenicity of and the Presence of Phytotoxins in Fusarium Fungi-the Pathogens of Root Rot of Wheat,'' Mikologiya i Fitopatologiya, Vol. 14, No. 1, 1980, pp. 51-56.

Kostyunina, N. A., Ermakov, V. V., ''Isolation and Identification of Zearalenone,'' Veterinariya, Moscow, USSR, No. 11, 1976, pp. 101-103.

Palyusik, M., Matrai, T., ''Effect of Carbon Dioxide and Mositure on Toxin Production by Fusarium Strains During Storage of Maize,'' Magyar Allatorvosok Lapja, Vol. 32, No. 9, 1977, pp. 572-573.

D-29

UNCLASSIFIED

Approved for Release: 2015/01/05 C05184036

Seelyey, G., Vanyi, A., Petri, A., ''Effect of Irradiation on the Viability and Toxin Porduction of Different Fungus Species,'' Magyar Allatorvosok Lafja, Vol. 34, No. 6, 1979, pp. 412-416.

Polovinki, G. P., ''Accumulation of Fusaric Acid by Different Fusarium Species and Their Phytotoxic Properties,'' Mikrobiologicheskii Zhurnal, Vol. 41, No. 5, 1979, pp. 504-508.

Bilai, V. I., Ellanskaya, I. A., ''Morphological Features of the Fusarium Species Under Submerged Cultivation,''Mikrobiol. Zh., Vol. 42, No. 2, 1980, pp. 172-179.

Maksimova, R. A., Rapoport, I. A., ''Investigation of the Mutagenic Effects of Nitrosomethylurea on Trichothecium Roseum, A Producer of the Antibiotic Trichothecin,'' Genetika. Publ. 67, Issue 3, pp. 107-113.

Zaichenko, A. M., Proskuryakova, N. S., Dakhnovsky, V. I., ''Physiological and Biochemical Characteristics of Dendrodochium Toxicum in Connection with Biosynthesis of Dendrodochins in the Process of Dendrodochium Toxicum 5800 Cultivation. II. Dynamics of Dendrodochins Component Composition,'' Mikrobiol. Zh., Vol. 42, No. 3, 1980, pp. 315-318.

Bilai, V. I., Nikol'skaya, E. A., Bogomolova, L. A., Zakordonets, L. A., Strizhevskaya, A. Ya., Silvers, V. S., Zaichenko, A. M., Proskuryakova, N. S., ''Regulation of Growth and Biosynthetic Activity of Microscopic Fungi,'' Mikrobiol. Zh., Vol. 33, No. 2, 1971, pp. 134-140.

Bilai, V. I., Shcherbina, S. N., Bogomolova, L. A., Proskuryokova, N. S., ''Effect of Different Ratios of Carbon and Nitrogen on Biosynthesis of Fusaric and Nicotinic Acids and on Respiration in Fusarium (Ukrainian),'' Mikrobiol. Zh., Vol. 36, No. 3, 1974, pp. 293-299.

Bilai, V. I., Koval, E. Z., ''Features of Growth of Fusaria With Assimilation of Hydrocarbons (Ukrainian),'' Mikrobiol. Zh., Vol. 36, No.5, 1974, pp. 587-594.

Bilai, V. I., Losjakova, L. S., Serebrennikov, V. M., Shkolnyj, A. T., ''Growth of Microbiol. Zh., Vol. 40, No. 3, 1978, pp. 327-332.

Bilai, V. I., Strizhevskaja, A. IA., ''Growth of Mycelium and Rate of Xylan, Xylose and Glucose Uptake By Strains of Different Fungi Species,'' Mikrobiol. Zh., Vol. 39, No. 3, 1977, pp. 307-310.

> D-30 UNCLASSIFIED

Panozishvili, K., Zol'nikova, N. V., Borovkov, A. V., ''Verrucarin A From Dendrodochium Toxicum,'' Khim. Prir. Soedin., No. 2, 1972, p. 245.

Kurbatskaya, Z. A., Ibragimov, R. G., ''Growth and Toxin Formation in Some Species of Aspergillus Genus Under Different Temperature Conditions of Cultivation,'' Mikrobiol. Zh., Vol. 41, No. 4, 1979, pp. 358-362.

Bilai, V. I., Shcherbina, S. M., Ellanska, I. A., ''Formation of Group B Vitamins by Different Species of Fusarium LK,'' Mikrobiol. Zhur., Vol. 33, No. 3, May/June 1971, pp. 310-314.

Bilai, V. I., ''Determination of Growth and Biosynthetic Activity of Fungi,'' Metody Eksp. Mikol., 1973, pp. 5-16.

Bilai, V. I. Misyurenko, I. P., ''Formation of Toxins During the Submerged Cultivation of Fusarium Sporotrichiella,'' Dopov. Akad. Nauk Ukr. RSR, Vol. 36, No. 9, 1974, pp. 846-849.

Bilai, V. I., Nikol'skaya, E. A., Bilai, T. I., ''Regulation of Enzymic Activity of Fungi by the Relation and Level of Nutrient Components,'' Fiziol. Puti Povysh. Ferment. Akt. Mikroorg., 1973, pp. 134-148.

Erofeev, N. S., Bezborodov, A. M. Bilai, V. I., Bogomolova, L. A., Zolotareva, E. A., Medvedeva, T. N., Surovtseva, V. M., Chermenskii, D. N., Fusaric Acid Patent, Institute of Biochemistry and Physiology of Microorganisms, Academy of Sciences, USSR, Patent SU 516739, 5 June 1976.

Zaichenko, A. M., Dakhnovskii, V. I., ''Growth Regulation and Toxin Formation of Dendrochium Toxicum and Stachybotrys Alternans by the Carbon/Nitrogen Nutrient Ratio,'' Tr. S'ezda Mikrobiol. Ukr., 1975, pp. 57-58.

Bilai, V. I., Zakordonets', L. A., ''Dynamics of Amino Acid Content in Fusarium Moniliforme 51070 in the Process of Growth,'' Mikrobiol Zhur, Vol. 33, No. 3, May/June 1971, pp. 306-309.

Bilai, V. I., Zakordonets', L. A., Shcherbina, S. M., ''Effect of Autolysis on Yield of Amino Acids and Group B Vitamins in Fusarium Moniliforme,'' Mikrobiol Zhur, Vol. 34, No. 3, May/ June 1972, pp. 292-296.

D-31

UNCLASSIFIED

Approved for Release: 2015/01/05 C05184036

Proskuriakova, N. S., Dakhnovs'kyi, V. I., ''On Some Morphological and Cytological Changes in Microscopic Fungi When Using Dendrodochine as a Substrate,'' Mikrobiol. Zhur., Vol. 34, No. 3, May/June 1972, pp. 297-300.

Bilai, V. I., Shematiuk, E. G., ''Cellulose-Digesting Mycomycetes of Some Soil Types in the Ukrainian SSR,'' Mikrobiol Zh., Vol. 36, No. 3, May/June 1974, pp. 300-303.

Bilai, V. I., Zakordonets, L. A., Bogomolova, L. A., Meteiko, T. IA., ''Effects of Different Ratios of Carbon and Nitrogen in a Nutrient Medium on the Growth and Metabolism of Fusarium Moniliforme Scheld. (Strain 2801),'' Mikol Fitopatol, Vol. 8, No. 3, 1974, pp. 177-184.

Ponozishvili, K. P., Borovkov, A. V., ''Roridn A From Dendrodochium Toxicum Fungi,'' Khimiia Prirodnykh Soedinenii, Vol. 3, May/June 1974, pp. 404-405.

Fafurova, V. L., Kurbatskaia, Z. A., ''Study of Toxin-forming Capacity of Some Species of Entomopathogenic Fungi (in View of Biologic Control),'' Izvestiia. Akademiia Nauk Tadzhikskoi SSR., Vol. 2, April/June 1978, pp. 28-34.

Bilai, V. I., Shcherbina, S. M., ''Effect of Concentration and Correlations of Sources of Carbon and Nitrogen on the Formation of Vitamins of Group B in Fusarium Moniliforme Sheld v. Lactis,'' Mikrobilogicheskii Zhurnal, Vol. 42, No. 5, September/October 1980, pp. 576-581.

Bilai, V. I., Ellanskaya, I. A., ''Microculture Method for Obtaining Typical Conidial Production in Fusarium spp.,'' Mikologiya i Fitopatologiya, Vol. 9, No. 1, 1975, pp. 74-76.

Bilai, V. I., ''Principles of the Systematics and Structure of Plant Pathogenic Species of Fusarium Lk. ex Fr.,'' Mikrobilogicheskii Zhurnal, Vol. 40, No. 2, 1978, pp. 148-156.

Bilai, V. I., Shoherbina, S. M., ''Dynamics of the Vitamin Content of Different Strains of Fusaria,'' Mikrobiologichnnii Zhurnal, Vol. 39, No. 5, 1977, pp. 597-600.

Ermakov, V. V., Kostyunina, N. A., Kurmanov, I. A., ''Isolation and Identification of T-2 Mycotoxin Produced by Fusarium Sporotrichiella,'' Doklady Vsesoyuznoi Akademii Sel'skokhozyoistvennykh Naulk, No. 3, 1978, pp. 36-38.

D-32



Kotik, A. N., Chernobai, V. T., Komissarenko, N. G., Trufanova, V. A., ''Isolation of the Mycotoxin of Fusarium Sporotrichiella and a Study of Its Physico-chemical and Toxic Properties,'' Mikrobiologicheskii Zhurnal, Vol. 41, No. 6, 1979, pp. 636-639.

Bilai, V. I., Zaichenko, A. M., Kirillova, L. M., Dokhnovskii, V. I., ''Physiological and Bichemical Features of Dendrodochium Toxicum in Connection With the Biosynthesis of Dendrodochines. I. Some Features of Dendrodochium Toxicum 5800 Growth,'' Mikrobiologicheskii Zhurnal, Vol. 42, No. 2, 1980, pp. 180-184.

Silaev, A. B., Bekker, Z. E., Maksimova, R. A., Gus'kova, T. M., Karnaukhova, M. V., Zeleneva, R. N., Paramonov, N. Ya., ''Trichothecin, Conditions of Its Biosynthesis and Isolation,'' S-Kh. Biol., Publ. 66, Series 1, Issue 4, pp. 627-631.

Maksimova, R. A., Pal'mova, N. P., Alekseeva, A. A., ''Effect of Vitamins on the Growth and Development of Various Strains of Trichothecium Roseum Producing Trichothecin,'' Antibiotiki, Publ. 70, Series 15, Issue 3, pp. 229-232.

Maksimova, R. A., Khuratova, B. Silaev, A. B., ''Synthetic Medium for the Tricothecin in Biosynthesis,'' Biol. Nauki, Publ. 71, Series 14, Issue 4, pp. 138-141.

Akhmedova, A. N., Velikanov, L. L., Sidorova, I. I., ''Effect of Absorbents on the Biosynthesis of Trichothecin by Trichothecin Roseum Strains,'' Vstn. Mosk. Univ., Biol., Pochvoved, Publ. 71, Series 26, Issue 4, pp. 49-51.

Bilai, V. I., Shkurenko, V. A., ''Effect of Temperature and pH on the Proteolytic Activity of Fungi,'' Fermenty Med., Pishch. Prom. Sel. Khoz., 1968, pp. 190-192.

Bilai, V. I., Kharchenko, S. M., Lemeshchenko, G. P., ''Toxicity of Dendrodochin in Relation to Nutritional Source of Dendrodochium Toxicum,'' Mikrobiol. Nar. Gospod. Med., Mater. Z'izdu Ukr. Mikrobiol. Tov., 1966, pp. 142-145.

Bilai, V. I., Kharchenko, S. M., ''Effect of Nutritional Source on the Growth of Dendrodochium Toxicum and the Antibiotic Properties of Dendrodochin,'' Mikrobiol. Nar. Gospod. Med., Mater. Z'izdu Ukr. Mikrobiol. Tov., 1966, pp. 138-141.

> D-33 UNCLASSIFIED

Bilai, V. I., Kharchenko, S. N., ''Physiology of the Growth of Dendrochium Toxicum and Formation of Dendrochin,'' Eksp. Mikol., 1968, pp. 97-105.

Paper submitted at symposium on mycotoxins held September 28 to 30, 1977 in Orenburg, ''Questions Related to the Biosynthesis of Steroid Mycotoxins,'' Reference MIFIB 78, Vol. 12, No. 3, p. 269.

Paper submitted at symposium on mycotoxins held September 28 to 30, 1977 in Orenburg, ''The Physiologic Bases of Regulation of the Processes of Fungal Toxin Formation,'' Reference MIFIB 78, Vol. 12, No. 3, p. 269.

''The Effect of Inhibitors of Protein Synthesis on the Growth and Biosynthetic Activity of Trichothecium Roseum,'' Reference MIKBA 79, Vol. 48, Nol. 5, pp. 858-862.

''Growth of Fungi of Different Genera on Mineral Medium With Lignin of Pinus Silvestris,'' Reference MZUKA 77, Vol. 39, No. 6, p. 740.

''Effect of an Addition of Enzymic Inhibitors and Stimulants to the Nutrient Medium on the Fibrinolytic and Antibiotic Activity of the Fungus Trichotecium Roseum,'' Reference PBMIA 77, Vol. 13, No. 4, pp. 515-520.

''Activator Properties of Protease Synthesized by the Saprophyte Fungus Trichothecium Roseum LK EX FR,'' Reference PBMIA 77, Vol. 13, No. 3, pp. 398-404.

''Kinetics of Hydrolysis of N Tolune Sulphonyl L Arginine Methyl Ester and n Benzoyl L Arginine Ethyl Ester Catalyzed by Tricholysine From Trichothecium Roseum,'' Reference PBMIA 77, Vol. 13, No.2, pp.. 241-247.

''Fungi From the Genus Trichothecium Link and the Antibiotic Substances Formed by Them,'' References MIKBA 77, Vol. 46, No. 1, p. 112.

''Procedure for Obtaining Trichothecin,'' Reference OIPOB 528918, 76, No. 35, p. 8.

''Natural Variability of Trichothecium Roseum,'' Reference MIKBA 77, Vol. 46, No. 1, pp. 109-112.

D-34

UNCLASSIFIED

Approved for Release: 2015/01/05 C05184036

''Effect of Colchicine and Other Polyploidogenous Factors During Contact With Submerged Vegetative Mycelium of Trichothecium Roseum, A Fungus Producing Antibiotic Trichothecin and Proteolytic Enzymes,'' Reference MIKBA 77, Vol. 46, No. 1 pp. 80-85.

''Morphogenetic Action of Trichothecin on Trichothecium Roseum,'' Reference MIKBA 76, Vol. 45, No. 6, pp. 1023-1027.

''A Procedure for Obtaining Fusaric Acid,'' Reference OIPOB 516739, Vol. 76, No. 21, pp. 78-99.

Ermakov, V. V., Kostyunina, N. A., Kurmanov, I. A., ''Isolation and Identification of Mycotoxin T-2 Produced by Fusarium Sporotrichiella,'' Soviet Agricultural Sciences, 1978, No. 3, pp. 47-49.

Bilai, V. I., ''Principles of Taxonomy and Structure of Phytopathogenic Species of the Genus Fusarium Lk. Ex Fr,'' Mikrobiol. Zh., Vol. 40, No. 2, 1978, pp. 148-156.

Bilai, V. I., Cherkes, A. I., Bogomolova, L. A., Frantsuzova, S. B., Toxicobiologic Properties of Fusaric Acid, A Metabolite of Fusarium Oxysporum (Ukrainian),'' Mikrobiol. Zh., Vol. 37, No. 3, 1975, pp. 325-328.

Palmova, N. P., Maximova, R. A., ''Effect of Inhibitors of Protein Synthesis on the Growth and Biosyntetic Activity of Trichothecium Roseum,'' Mikrobiologiya, Vol. 48, No. 5, 1979, pp. 858-862.

Maximov, V. N., Maximova, R. A., Minayeva, T. A., ''Characteristics of the Directed Biosynthesis of Trichothecin and Fibrinolytic Enzymes in Trichothecium Roseum,'' Mikrobiologiya, Vol. 49, No. 2, 1980, pp. 258-264.

Bilai, V. I., Olifson, L. E., ''Mycotoxins (Producers, Chemistry, Biosynthesis, Determination, Effect on the Body),'' Izv Akad Nauk SSSR Biol., Vol. January/February 1979, pp. 150-155.

Olifson, L. E., Kenina, S. H. M., Kartashova, V. L. ''Fractional Composition of the Lipid Complex of Grain Infected by the Microscopic Fungus Fusarium Sporotrichiella Bilai,'' Prikl Biokhim Mickrobiol., Vol. 14, No. 4, July/August 1978, pp. 630-634.

D-35

Bilai, V. I., ''Principles of the Systematics and the Structure of Phytopathogenic Secies of the Genus Fusarium Lk. Ex Fr,'' Mikrobiol Zh., Vol. 40, No. 2, March/April 1978, pp. 148-156.

Olifson, L. E., ''On the Question of Biosynthesis of Toxic Steroles by the Microscopic Fungi Fusarium Sporotrichiella, Paper Presented,'' Mikrobiologichnyy Zhurnal, Vol. 35, No. 2, 1973, p. 266.

Dunin, M. S., Prasad, Y., ''Effect of Zinc Upon Growth of Fusarium Oxysporum F. Inli (Bolley) Synder Et Hansen and the Formation of Toxins,'' Izvestiya Timiryazevskoy Selskokhozyaystvennoy Akademii, No. 3, 1972, pp. 143-147.

Alisova, Z. I., ''Immunization of Rabbits by the Toxic and Atoxic Condensation of Liquid Cultures of the Fungus Fusarium,'' Voprosy Meditsiny, Vol. No. 1 1964, p. 190.

Kvashnina, Ye. S., ''Water Soluble Toxic Substances From Fusarium Genus Molds (4th All Union Conference on Aerosols, 1958)'' Letopis Zhurnalnykh Statey, No. 38, 1959, p. 127.

Titova, L. M., ''Amino Acids as a Single Source of Nitrogen for Toxin Forming Fungi,'' Mikrobiologichnyy Zhurnal, Vol. 33, No.2, 1971, pp. 159-164.

Bekker, Z. E., Poletayeva, V. F., ''The Role of Zinc in the Pathogensis of Fusarium Wilt and the Biosynthetic Activity of Strains of Its Etiologic Agent,'' Izvestiya Akademii nauk Turkmenskoy SSR, No. 2, 1968, pp. 3-9.

Gubin, I. Ye., ''Morphological and Culture Properties of Some Toxic Fungi of the Genus Fusarium,'' SB Nauchn Rab Ryazan S-Sk Inst, Vol. 24, No. 2, 1970, pp. 51-55.

Gubin, I. E., ''The Development of Toxic Strains of Fusarium Sporotrichiella Bilai Var Sporotrichioides, Sherb, Bilai Under Different Conditions of Cultivation,'' SB Nauch TR Ryazan Sel'skokhoz Inst, Vol. 17, 1967, pp. 172-175.

Kurbatskaya, Z. A., ''The Effect of Certain Factors on Toxin Formation in Fish of the Species Fusarium,'' Letopis Zhurnalnykh Statey, No. 32, 1969, p. 140.

> D-36 UNCLASSIFIED

Bekker, E. E., Doviet, M., Ovletmuradov, K. D., Pushkareva, I. D., Poletayeva, V. F., Shilina, S. G., Yaskova, E. I., ''Nature and Biosynthesis of the Causative Agent Toxin of Fusariosis Wilt, the Mechanism and Its Action and Possible Transformation Within the Organism of the Cotton Plant,'' Izvestiya Akademii Nauk SSSR, No. 5, 1971, pp. 749-754.

Bilai, V. I., Pidoplichko, N. M., ''Toxin Producine Micromycetes,'' Izvestiya Akademii SSSR, No. 4, 1970, pp. 600-608.

Guntaishvili, R. K., ''Material About the Study of the Stimilant of Red Mold of Corn in Georgia,'' Soobshcheniya Akademii Nauk Gruzinskoy SSR, Vol. 32, No. 1, 1963.

Hoshayev, M. H., Hubin, I. E., Leonov, A. N., Shylina, S. H., Soboleva, N.A., ''Toxin Forming Characteristics of Certain Types of Fungi of the Fusarium Family,'' Mikrobiologichnyy Zhurnal, Vol. 35, No. 2, 1973, p. 266.

Pidoplichko, V. M., ''Toxicity of Fungi From the Genus Fusarium Agents of Root Rot in Winter,'' Mikrobiologichnyy Zhurnal, Vol. 32, No. 6, 1970, pp. 700-704.

Akhmeteli, M. A., Linnik, A. R., Chernov, K. S., Voronin, V. M., Khesina, A. Ya., Guseva, N. A., Shabad, L. M., ''Toxins Isolated Form Grain Infected With Fusarium Sporotrichioides,'' Pure and Applied Chemistry (London), No. 35(3), 1973, pp. 209-215.

Prasad, Yogendra, ''Zinc in the Toxin Motabolism of Fusarium Oxypsporum,'' Indian Journal of Agricultural Science (New Delhi), No. 42(10), 1972, pp. 950-952.



ANNEX E

MEDICAL EVIDENCE

Southeast Asia

Since 1976, multiple kinds of sources--refugees, relief medical and civilian workers, and many specially qualified physicians--consistently have detailed unusual signs and symptoms of victims of 'yellow rain.'' Specifically, victims in Southeast Asia subjected to a direct attack of the yellow powder, mist, smoke, or dust would be seen to begin retching and vomiting in a matter of minutes. This and the effects described below were not pronounced in individuals even 100 meters from the attack zone, indicating a relatively dense chemical/carrier combination that was effective in low wind conditions.

Unlike that caused by a traditional riot-control nausea agent, the initial induced vomiting following exposure to ''yellow rain'' was protracted over hours to days often accompanied by dizziness with rapid heartbeat and apparently low blood pressure, chest pain, loss of far-field vision, and a feeling of intense heat and burning on the skin, but not described as being most acute in the groin and axillae. Thus, the acute signs and symptoms match some of the effects of traditional vomiting and blister agents, but clearly not all.

A significant number of victims also reported intense ''red eyes,'' ''bleeding gums,'' convulsions or more often trembling, and vomiting of blood with or without production of copious amounts of saliva within the first hours after the attack again lasting many hours to days apparently depending on the exposure level. Thick mucus, pinpoint pupils, respiratory collapse, prolonged spasticity, and involuntary urination or defecation were never reported after a yellow rain attack, and this absence helped to rule out organophosphate nerve agents in the minds of CW experts. Many-medical and environmental samples also ruled out these and other traditional agents such as DM, DS, and others.

Many observers of the ''yellow rain'' effects reported formation within several hours of small (1 centimeter) homogeneous, hard, fluid-filled blisters over only exposed areas of skin, frequently including the victim's hands, arms,

E-1

entire throat, and face, whenever skin was uncovered. In most cases, the vomit, after two to eight hours, contained blood, and in many cases a good deal of it. About half of those receiving the most concentrated doses of yellow material who had been directly under the spray were in several hours after the attack observed to cease vomiting tempo-This interval was often followed in five to 15 rarily. minutes by a period of great pain when the victim would hold his abdomen and emit a ''gush'' of blood from his mouth and nose. These individuals were usually dead within minutes after that. Close questioning by physicians of those who witnessed these final moments leave no doubt that these observed effects were the results of severe gastrointestinal bleeding, significant pulmonary bleeding, temporary compression of accumulated blood in the stomach, and finally projectile vomiting of as much as several hundred milliliters of blood. These findings were consistent with animal and human autopsies.

Many victims of the yellow material received less than the full brunt of a spray, or entered the attack zone several hours to two days later, or consumed food or water contaminated by the material. These individuals often within the next 24 hours developed signs and symptoms similar to those of the more directly affected (but often without pronounced skin effects if they did not contact the powder residue directly). In addition to attacks of intense vomiting (five or six times a day), they also had diarrhea, with bloody stools passed up to eight times a day. Bleeding under the fingernails and around the skin of the eyes and severe bruising of the skin are also commonly reported. Opiates helped the fluid loss in adults; but, in children or young persons not able to tolerate the treatments of raw opium and water, death occurred in about half the cases after 10 days to two weeks. On the basis of reported signs and symptoms, the cause of delayed death was almost certainly dehydration.

In many cases chemical attacks are reported to produce symptoms other than the ones described here. There has always been, however, a direct association of the symptoms above with reports of ''yellow rain'' attacks -- that is, when yellow material is used these symptoms appear; other agents may give rise to other symptoms. Although it is possible to have one or even several of these symptoms associated with

E-2

Approved for Release: 2015/01/05 C05184036 UNCLASSIFIED

traditional CW agents, no expert has been able to ''fit'' the sequence, severity, and consistency with any of them. In many cases victims and observers were examined, histories taken, and interviews conducted by several health professionals weeks apart. Remarkable consistency has been observed.

From the beginning of the ''yellow rain'' episodes in 1976, autopsies have occasionally been reported anecdotally. Some have been done by less than expert technique, some by nonphysicians, and some were on animals rather than human victims. However, the consistency of the early reported ''putrefaction'' or ''rotteness'' of the digestive tract within 12 to 48 hours after death led many forensic experts to suspect that one effect of the poison, whatever it was, was to cause necrosis (cell death) of rapidly dividing mucosa (mucous membranes), especially in the stomach and upper small intestine. Other autopsy findings included hyperemia (engorgement with blood) of digestive mucosal linings, and remarkably intense congestion and swelling in the lungs, liver, spleen, and sometimes the kidneys. These and other findings often led experts in toxicology and pathology to suggest mycotoxin or even trichothecene intoxication based on clinical and pathological data alone.

Although not a common cause of death, trichothecene effects have been reported in the forensic, oncological, and toxicological literature for several years. Unpublished findings often were discussed in symposiums. In several dozens of cases toxic effects in humans and animals have been carefully recorded, and they match those of the ''yellow rain'' story with good precision. (See table.) There are no additional signs or effects of known trichothecene intoxication not frequently reported by victims, nor are there any reported ''yellow rain'' symptoms that cannot be explained by the effect of the four specific trichothecene toxins found in the samples, and the doses inferred from the operational situation and description.

From a medical viewpoint, there are no significant differences in the reporting from Laos and Kampuchea. The timing and delivery systems have sometimes varied, but the effects of the chemical agent, both clinically and pathologically, are identical. In one case, a series of blood samples from Kampuchean victims also showed a trend toward leukopenia (reduction in number of white blood cells) and the presence of a trichothecene metabolite (HT-2) consistent

E-3

Comparison of Reported ''Yellow Rain'' Effects With Known Trichothecene Effects

Yellow Rain Reports

- 1. Nausea, vomiting--severe, immediate
- 2. ''Falling down, world turning''
- 3. ''Burning of skin'' . . . small blisters
- 4. ''Shaking all over, flopping like fish out of water''
- 5. ''Bleeding eyes''
- ''Pounding'' chest, rapid heartbeat, weakness
- 7. Severe pain in center of chest
- 8. Sleepiness, ''not able to talk''
- 9. Bleeding gums
- 10. ''Can't breathe''
- 11. ''Skin and body hot with cold''
- 12. Diarrhea with blood
- 13. Loss of appetite, inability to eat
- 14. Bleeding into skin and fingernails
- 15. Drop in white blood cell count
- 16. ''Rotten esophagus, stomach, intestines; soft spleen and liver''
- 17. Swelling of all organs

Effects of Trichothecenes (*)

(C)

- 1. Nausea, vomiting--severe, immediate
- 2. Dizziness
- 3. Generalized erythema with a burning sensation of skin
- 4. Ataxia (failure of muscular coordination), occasional tremors and convulsions
- 5. Congestion of the sclera (white outer coat of eyeball) and blood in tears
- 6. Hypotension (abnormally low blood pressure) with secondary rise in heart rate
- 7. Angina (substernal chest pain)
- 8. Somnolence, central nervous system symptoms
- 9. Stomatitis (inflammation of oral mucous membranes) and ptyalism (excessive salivation)
- 10. Shortness of breath
- 11. Fever and chills
- 12. Diarrhea with blood
- 13. Anorexia
- 14. Thrombocytopenia (decrease in number of platelets, white blood cells involved in clotting of blood) and purpura (skin discoloration caused by hemorrhage into tissues)
- 15. Leukopenia and anemia
- 16. Rapid necrosis of linings of gastrointestinal tract; lymphoid necrosis in spleen and liver
- 17. Congestion of all organs

^{*} Effects are immediate at levels near to or above 500 to 1,000 mg total body burden for an adult. Athough inhalation data are pending, the levels are consistent with reported lethal and sublethal doses. Trichothecenes in combination when directly ingested or inhaled are more toxic in some cases, and the order of signs and symptoms and timing varies.

with trichothecene intoxication (see annex D for blood analysis results). To a first order, dose-response effects are also seen, and routes of administration are consistent with effects.

Public Health Issues. An early hypothesis (1978-79) was that a significant number of the deaths, especially in Laos, could be explained by the heavy use of riot-control agents such as CS, CN, DM, and agents which cause itching and/or blistering. The hypothesis was rejected quickly on two grounds. First, trace contaminant analysis did not show the presence of any of these compounds in samples (several samples did, however, contain a trichothecene precursor). Secondly, contrary to commonly held views, the epidemiology of diseases endemic to the Central Highlands and the public health of the H'Mong do not support the view of malnourished, disease-ridden, and weak persons who would succomb easily to riot-control agents. Also, a number of studies have shown the opposite: a relatively low incidence of pulmonary disease (lower than what could otherwise account for certain effects); better nutritional states than could otherwise account for death in 10 days to two weeks from water loss (dehydration) and calorie depletion; and a death rate of near zero from causes other than infection, gerontological causes, and trauma.

Afghanistan

Some deaths with bleeding have been described in the accounts from Afghanistan. In one series of cases a physician examined a number of persons who had been exposed to sublethal doses of a yellow smoke/black smoke combination attack and one man near death after a series of attacks. Hemoptysis (nasal bleeding)--but not hematemesis (bleeding from the gastrointestinal tract)--was reported in about half of these and other cases.

Several features of at least one of the chemical agents-an incapacitant--used in Afghanistan defy explanation at this time. One posture is that it is highly selective for the central nervous system rather than the autonomic nervous system. No good candidate has yet been identified which will selectively inhibit the central nervous system to cause unconciousness for several hours as reported. A second finding has been the presence of a dermal anaesthesia, affecting only exposed areas of skin.

E-4

UNCLASSIFIED

Postattack Medical Survey. There is evidence that after some of the attacks in Laos and Afghanistan, Soviet or Communist (Pathet Lao) forces entered the attack zones to conduct surveys. Several reports indicate that a group of survivors from a toxin attack on a Laotian village were taken several kilometers from the village and injected with a small volume of a clear solution said by their captors to be a ''new'' medicine to ''assess the gas.'' The injections were given intramuscularly in the upper arm and reportedly did nothing to alleviate the weakness, nausea, vomiting, or diarrhea suffered by the survivors. One source reported the effect of the drug was to cause an immediate sensation of warmth throughout his body. Only the use of opium later eased the discomfort, after the survivors had lied to their captors about how much better the new medicine made them feel. It is probable that the procedure was a test, either of a new antidote or of a drug developed to reduce incapacitation from the nausea and vomiting.

Similarly, in a few cases in Afghanistan, Soviet troops were reported to disembark from helicopters or armored personnel carriers at the edge of an attack site. Three or four, dressed in full anticontamination gear, walked among the dead; examined the corpses; and, opening them with a crude autopsy incision, examined the organs in the abdominal and thoracic cavities. In one report a solution was poured into the incision. When the corpses were later recovered by Mujahedin guerrillas, the body cavity contents were destroyed beyond recognition. These bizarre stories would be discounted were it not for the past reliablility and quality of reporting from the sources, which is believed excellent.

These and a small number of additional reports support the hypothesis that the perpetrators of some of the attacks are interested in studying aftereffects, lethality, or some other quasi-experimental aspect of use of a new chemical weapon. Details are not sufficiently clear to explain the purpose of the above events, and ''destruction of evidence'' is by itself not an entirely credible explanation. Recent indications from Afghanistan indicate that one purpose of these reviews of bodies and the field surveys is to determine levels of toxic materials still present in the attack zone before Soviet troops occupy it.

E-5